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STUDIES ON LIGNIN AND RELATED COMPOUNDS. VIII. A KINETIC STUDY OF THE ACTION OF SULPHUROUS ACID ON LIGNIN AND RELATED COMPOUNDS[1]

BY CHARLES A. SANKEY[2] AND HAROLD HIBBERT[3]

Abstract

Electrical conductivity measurements have been made over the temperature range 18-145°C. of reaction mixtures of sulphurous acid and a large number of type compounds, and data obtained on the stability of the resulting sulphonic acids and the mobility of the equilibria involved. The compounds investigated include saturated and unsaturated aldehydes; saturated, unsaturated and cyclic ketones; phenols; substances in which an ethylene linkage is the only reactive group; furane derivatives; and substances containing pyrone rings.

The results are discussed from the standpoints of structure and reaction mechanism. The sulphonic acids of nuclear aldehydes, saturated ketones and cylic ketones are typically unstable. With compounds containing an ethylene linkage conjugated with a carbonyl group, addition normally takes place in the 1:4 positions and is followed by hydrogen migration. Where an ethylene linkage is the only reactive group present, addition proceeds only very slowly and at high temperatures. The difficulty of effecting a reaction between sulphurous acid and phenols in their tautomeric alicyclic forms is indicated by the non-reactivity of resorcinol, and the formation of only a small percentage of an extremely unstable derivative by phloroglucinol. Furfuryl alcohol and glucal are shown to form oxonium derivatives which serve as intermediates for the entry of the sulphonic acid group into the ring system.

Conductivity-temperature curves for lignosulphonic acids from various sources indicate that the products are sulphonic acids of the $-C=C-$type. Other evidence points to an oxonium addition product with a ring oxygen as being a probable intermediate in the formation of the final and more stable lignosulphonic acid. The relation of such a mechanism to the experimental conditions employed in the commercial process of the manufacture of sulphite pulp is indicated.

The formation of lignosulphonic acids through tautomerization of a phenolic nucleus is highly improbable. Also for such formation a carbonyl group does not necessarily have to be present. Strong additional evidence is presented for the presence of a heterocyclic ring containing oxygen and for an ethylene linkage in the lignin molecule. The latter is indicated to be in the same ring system as the oxygen atom.

[1] *Manuscript received February 10, 1931.*

Contribution from the Industrial and Cellulose Chemistry Laboratories, McGill University, Montreal, Canada, with financial assistance from the Canadian Pulp and Paper Association. Constructed from a thesis submitted to the Faculty of Graduate Studies and Research of McGill University by Charles A. Sankey, in May, 1930, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The first part of this study appeared in the Journal of Physical Chemistry (27).

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Introduction

The necessity for a kinetic as opposed to a purely organic chemical approach to the lignin problem has already been emphasized in these communications (25, 26, 27). The present investigation, which was commenced by Hoover and Hunten (27), had as its first object a kinetic study of the products of reaction of sulphurous acid with substances of known constitution containing chemical groupings similar to those which have been indicated as present in lignin, and as its second an extension of this study to the lignosulphonic acids.

The active part of the lignin complex in its reaction with sulphurous acid may be (a) an actual or potential carbonyl group; (b) an actual or potential ethylene linkage; (c) a heterocyclic ring containing an oxygen atom or atoms capable of oxonium salt formation; or (d) any combination of the above.

Evidence for the Presence of Various Groups in Lignin

Carbonyl Group

The presence of a carbonyl group has been indicated by the isolation by Friedrich and Diwald (7) of a condensation product of a specially prepared lignin with phenylhydrazine; by the further investigations of Friedrich (6), who claims to have established the existence of a tautomeric —CO—CH_2— group by isolation of two soluble forms of lignin which are interconvertible; and by the assumed formation of lignin acetals by Hägglund and Urban (20), who extracted wood meal with isoamyl alcohol using hydrochloric acid as a catalyst. Various polyalcohols have also been employed in this laboratory for the isolation of lignin (22, 23, 26), but there is some doubt as to whether the products are lignin acetals. The presence of active carbonyl groups has been assumed in the "lignin formulas" of Cross and Bevan (3), Schrauth (48), and Klason (33).

The majority of present workers however prefer to consider the carbonyl group as latent. Thus Fuchs (10) suggested that it may be formed by ketonization of a phenolic nucleus, while Klason in putting forward his coniferyl paraldehyde formula for lignin (34, 35) indicated a type of latency due to polymerization. Hägglund, Johnson and Trygg (19) postulated a similar reason for the assumed latency. Another possibility is that a carbonyl group is formed by loss of water from the molecule, followed by a keto-enol shift, as suggested by Freudenberg, Zocher and Dürr (5). A latent carbonyl group is also present in flavones and flavonols (18). The evidence for this latter type of structure will be discussed later.

Ethylene Linkage

Klason has assumed that in lignosulphonic acids the sulphonic acid group adds on directly to an ethylene linkage and that an aldehyde group is also present. This idea (30, 32, 33, 34, 35), culminating in the coniferyl paraldehyde formula already mentioned, is based on the presence of the glucoside coniferin in the cambial sap, and on his experiments with β-naphthylamine derivatives of lignosulphonic acids. The developments from the latter are practically pure speculation made in order to account for his analytical results, which are

not at all convincing. As Pauly and Feuerstein (43) have shown, some of Klason's experimental work is in serious error.

Better evidence is given by Fuchs (13), who claims that a glucal complex is present in lignin. More recently Fuchs and Horn (16) have shown that the action of bromine dissolved in carbon tetrachloride on an acetylated pine wood is analogous to that on a partially hydrogenated benzene nucleus. They claim that their data exclude the possibility of a larger ring, or a straight chain being involved, and therefore assume lignin to contain a tetrahydrobenzene ring. Reference has already been made to Friedrich's —CO—CH_2— tautomers (6).

On the other hand Rassow and Zickmann (46) state that lignin is unaltered by reduction with aluminium or zinc and acetic acid and is therefore saturated. It has been shown in Part V of this series (25) that dry chlorine has no action on dry spruce meal. This non-reactivity with halogens must be somewhat discounted however, since unsaturated compounds are known which will not react with bromine (50).

Ring Oxygen Atom Capable of Oxonium Salt Formation

The formation of oxonium derivatives by pyrones has long been established while a similar possibility for furane oxygen has recently been pointed out by Moureu, Dufraisse and Johnson (41). Those who consider lignin as being derived from the condensation of 2, 5-anhydroglucose units assume it to contain several five-membered heterocyclic rings containing oxygen. The formulas of Schrauth (48) and of Jonas (28) are derived on this basis. These rings have not a true furane character since they are saturated. The formula of Jonas does however contain an ethylene linkage. Under drastic experimental conditions 5-hydroxy-methylfurfural has been obtained from lignin (11, 12).

Rassow and Zickmann (46) insist on the presence of a pyrone ring stating that lignin contains the essential skeleton of a 2-phenyl flavone. Using Willstätter lignin, they claim that the removal of the dark color of the product on washing with water is due to the decomposition of an oxonium salt. A recent paper by Fuchs and Horn (17) also supports this view. They report that both HCl-lignin and acetyl-lignin react with glycol methyl ether in the presence of hydrochloric acid to yield a product containing three more free hydroxyl groups than were present in the original substance. Two of these hydroxyl groups are capable of acetal formation and the third is phenolic. The reaction is therefore claimed to be the transformation of a mixed aromatic heterocyclic derivative into a keto-hydroxy body. The heterocyclic grouping is also claimed to be present in genuine lignin but is much more readily subject to chemical action in this original form than in the isolated product, *e.g.*, it is much more readily acetylated.

The flavone type of structure would also account very well for combination between the carbohydrates of the cell wall and lignin (analogously to the anthocyanins), such as the ether or ester linkage between the polysaccharides and lignin postulated by Friese (8) or the glucal complex of Fuchs (13, 14), and would explain the almost limitless tendency of lignin to give color reactions.

The suggestion is also reasonable in view of the wide distribution of such products in plant life. On the other hand lignin can hardly be a simple flavone. These compounds yield well-defined hydrolysis products which indicate their constitution.

Previous Investigations

The first part of the present research, begun by Hoover, Hunten and Sankey (27), comprised measurements of the interaction products of sulphurous acid and certain aldehydes over the range of temperature normally used in the process of sulphite cooking, *viz.* 18-145° C. Provided that the data so obtained could be interpreted as a constitutive property of the original aldehyde, the investigation was to be extended to other compounds of known constitution and finally to the lignosulphonic acids themselves.

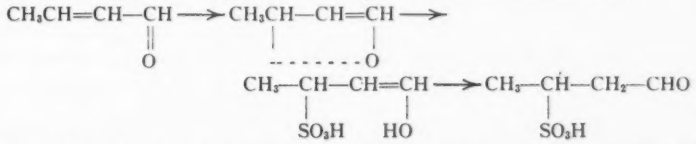
Electrical conductivity studies of lignosulphonic acids have been made previously by Melander (40) who titrated certain lignosulphonic acid fractions with sodium hydroxide and determined the equivalent weight by conductivity measurement, the conductivity passing through a minimum at the equivalent point. He also reported that the lignosulphonates were quite highly dissociated. Conductivity measurements were also made by König (37), who found that the acids were strong acids whose sodium salts were not hydrolyzed. Finally Kerp (29) studied the conductivity of formaldehyde sulphonic acid, and also of acetaldehyde sulphonic acid over a more limited range of conditions, and calculated the degree of dissociation for both. The figures for these, and also for the change of equivalent conductivity with dilution, presented fairly conclusive evidence that the compounds were strong acids.

Hoover, Hunten and Sankey (27) investigated the sulphonic acids of butyraldehyde, crotonaldehyde, cinnamic aldehyde and hydrocinnamic aldehyde. The apparatus described by them for making and handling the compounds in an atmosphere of nitrogen was used throughout the present investigation. The conductivity cell was a modification of Campbell's (2) instrument.

Measurements of specific conductivity were made for these systems over the temperature range 18-145° C. Two types of curves were obtained. (1) *From saturated aldehydes:* In these cases the conductivity rose with increasing temperature to a maximum at about 65-75° C. and then decreased up to the highest temperatures at which measurements were made. (2) *From unsaturated aldehydes:* With these, on first heating the reaction mixture in the conductivity cell, the specific conductivity followed the same course as for saturated aldehydes, but after passing through the usual maximum broke away and rose steeply at a temperature of 100-110° C., thereafter continuing to rise with increasing temperature. The path of the downward curve (taking readings with decreasing temperature) was not identical with the upward but showed no "breaks" and the conductivity fell continuously. On heating the cell a second time the downward curve was repeated. The character of the curve was taken as showing the presence of a sulphonic acid involving the ethylene linkage, *e.g.*, (I) or (II) from crotonaldehyde.

(I) $CH_3CH(SO_3H)CH_2CHO$ (II) $CH_3CH_2CH(SO_3H)CHO$

It should be noted that if the reaction involved essentially a 1:4 addition followed by hydrogen migration then (I) would be formed thus



while if the action was one of direct addition to the ethylene linkage both (I) and (II) would result.

It was further shown that at room temperature even in the presence of excess sulphurous acid, addition took place to the aldehyde group only, yielding $CH_3CH=CH—CH(OH)SO_3H$.

The reaction products were proved to be strong acids by their $A-\log D$ curves and therefore sulphonic acids ($R—CH(OH)—SO_3H$), and not sulphite esters ($R—CH(OH)—O—SO_2H$). This confirmed the conclusions of Stelling (49) based on X-ray analysis.

Systems Studied

Nuclear Aldehydes

The reaction of aldehydes in which the $-CHO$ group is connected directly to the benzene nucleus is of interest since Freudenberg, Harder and Markert (4) hold that a nucleus of the vanillin type is present in lignin, and Kürschner (38) claims to have obtained large yields of vanillic acid from Willstätter lignin by sublimation at 190-210° C. Two such aldehydes were therefore studied, namely, benzaldehyde and vanillin.

System:— Benzaldehyde—Sulphurous Acid

With this system, while a sulphonic acid is readily formed, it is much less stable than any of the other aldehyde sulphonic acids previously studied. Thus in approximately 0.1 *M* solution, for an equimolar reaction mixture, the equilibrium was about 92% on the side of the sulphonic acid at 20° C. but it is so readily shifted that when free sulphurous acid was determined by the usual iodometric method high results were obtained. To get accurate figures it was found necessary to freeze the equilibrium by running the sample into a very slight excess of iodine in a beaker containing ice and completing the titration as rapidly as possible.

The equilibrium is also shifted towards the left on dilution. Thus if a 0.1 *M* solution be diluted ten times, the sulphonic acid in the resulting solution will be less than 0.01 *M*. A comparison of the calculated dilution (0.1 *M* = 100 litres) with the true dilution (analysis = 116 litres) obviously measures the equilibrium shift. The values obtained for this sulphonic acid are shown in Fig. 1, curve *C*. The other curves in this figure indicate similar data for other sulphonic acids studied. A greater slope of the curve corresponds to increased decomposition of the sulphonic acid.

The ease of displacement of the equilibrium is also shown by the extreme

readiness with which the sulphonic acid is hydrolyzed by barium hydroxide. In the basic medium of excess of this base, free sulphurous acid is precipitated

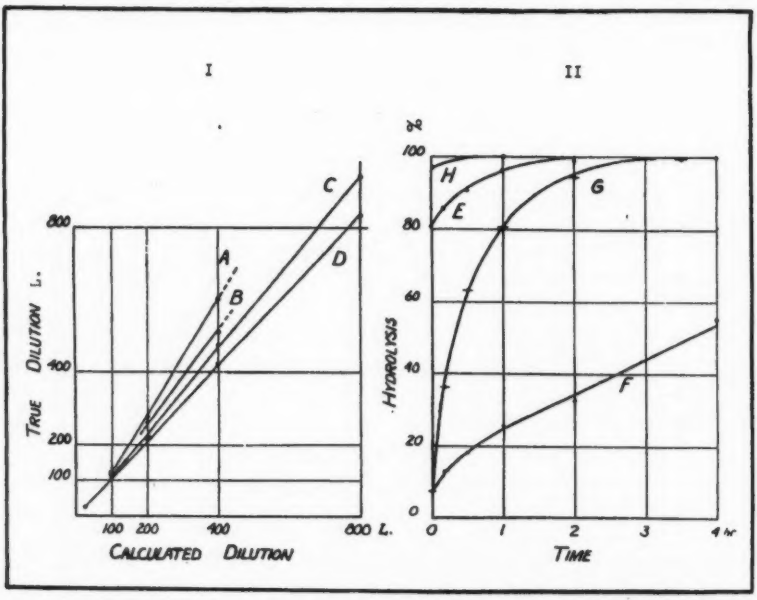


FIG. 1. *Effect of dilution on the equilibrium:* $R.CO.R' + H_2SO_3 \rightleftharpoons R.C(OH)SO_3H.R'$. *A. Methyl ethyl ketone (5×excess). B. Vanillin (equimolar). C. Benzaldehyde (equimolar). D. Cyclohexanone (2.5×excess). II. Hydrolysis of sulphonic acids with barium hydroxide. E. —CHO acid of crotonaldehyde (0.0329 M). F. —C=C— acid of crotonaldehyde (0.0336 M). G. Benzylidene acetone sulphonic acid (0.0328 M). H. Benzaldehyde sulphonic acid (0.1032 M).*

as barium sulphite and the equilibrium thus shifted. Provided that the reaction takes place in a nitrogen atmosphere to prevent the oxidation of sulphite to sulphate, the total sulphite may be readily determined at the end of a given time by acidifying the mixture and analyzing iodometrically, and the course of the hydrolysis thus followed. This method was used extensively later, as a measure of the stability of sulphonic acids. With benzaldehyde sulphonic acid, hydrolysis is over 95% complete almost immediately and 100% complete in about 10 min., a very rapid rate as shown by curve *H* of Fig. 1.

Conductivity measurements were made over the usual range of temperature at 229 litres dilution (sulphonic acid). The curve obtained is similar to that for vanillin as shown in Fig. 2. The type is that of the usual saturated aldehyde except that the down curve breaks slightly away from that obtained with rising temperature and indicates by its higher maximum an increased concentration of sulphurous acid. There is therefore at higher temperatures an apparently lasting decomposition of the sulphonic acid, in other words recombination does not occur to a sufficient extent to restore the

former equilibrium. A second conductivity run was made at the same dilution, with 110° C. as the maximum temperature. A similar type of curve was obtained showing to a somewhat lesser extent the sulphonic acid decomposition noted above.

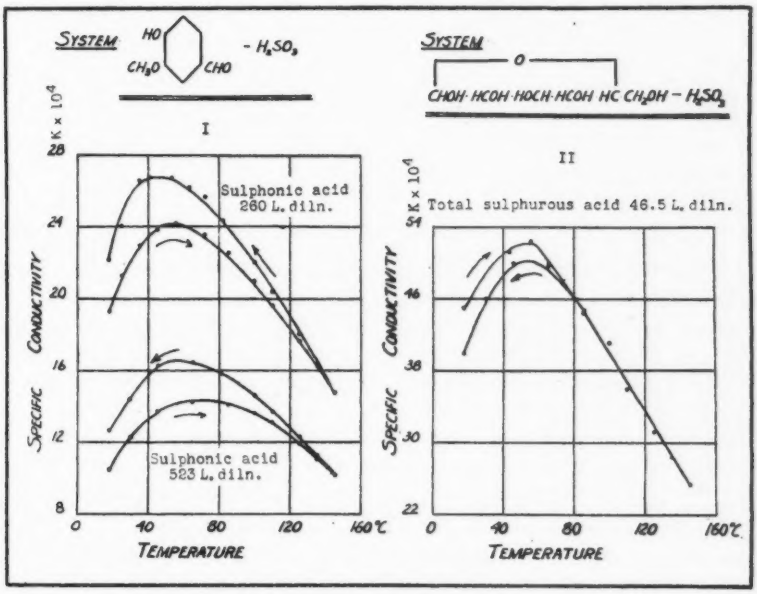


FIG. 2. *I. Specific conductivity of vanillin-sulphurous acid system.*
II. Specific conductivity of glucose-sulphurous acid system.

System:— Vanillin—Sulphurous Acid

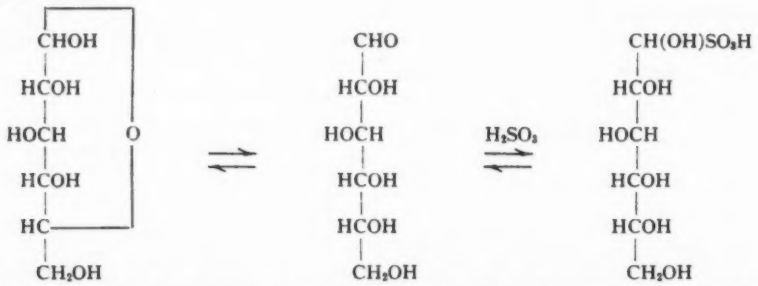
The characteristics of this system were found to be similar to those noted for the benzaldehyde reaction mixtures except that the sulphonic acid was even more unstable, probably due to the influence of the *p*-hydroxyl in the benzene ring. Thus curve *B*, Fig. 1, shows the equilibrium shift on dilution and, by its greater slope, indicates greater instability than in the case of benzaldehyde sulphonic acid. The rate of baryta hydrolysis is as rapid as that of the system previously discussed. The latter method is, of course, less sensitive for determining differences between acids that are easily hydrolyzable than between more stable ones.

Conductivity-temperature data were obtained at sulphonic acid dilutions of 260 and 523 litres over the 18-145° C. range (Fig. 2). As in the case of benzaldehyde one run was made with 110° C. as the maximum temperature. The curves showed the same high temperature decomposition of sulphonic acid.

System:— Glucose—Sulphurous Acid

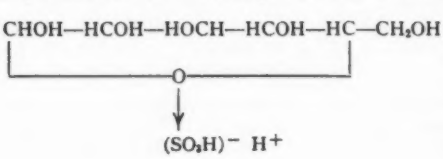
This system was selected in view of the essentially latent character of the

aldehyde group in glucose, since in solution the free aldehyde concentration is to be regarded as very low. The probable reactions involved are those shown in the scheme:



Analysis showed the reaction proceeded slowly. In equimolar solution (0.0770 *M*) only 6.5% of the total sulphurous acid combined in five days. The sulphonic acid percentage was raised to 14.9% by removing excess sulphur dioxide in vacuum. The conductivity-temperature curve, Fig. 2, indicates that the sulphonic acid is probably nearly all decomposed at temperatures over 60° C.

A possible alternative to the above mechanism was suggested by later experiments with furfuryl alcohol and with glucal (*q.v.*), namely, that the reaction product was an oxonium derivative (IIa). In the lack of other evidence however that glucose is capable of forming such compounds, the authors prefer to believe that the very slow realization of equilibrium concentration of the sulphonic acid is indicative of a mechanism of formation through an intermediate whose concentration in the reaction mixture is always low. This fact is of considerable interest as bearing on the cyclic-open chain glucose equilibrium in solution. It would seem to offer confirmatory evidence as to the probable presence of the free aldehyde form in only relatively small amount.



(IIa)

KETONES

The interaction of sulphurous acid with various types of ketones has also been investigated. The ketones studied were methyl ethyl ketone, benzylidene acetone, dibenzyl ketone and dibenzoyl methane.

System:— Methyl Ethyl Ketone—Sulphurous Acid

A study of the interaction of this typical saturated ketone and sulphurous acid emphasizes the characteristic difference between aldehyde- and ketone-sulphonic acids. The latter are much more unstable. The equilibrium is usually much further to the left than in the case of the corresponding aldehyde equilibrium and is also much more readily displaced. This is shown by the

equilibrium shift on dilution (Fig. 1, curve *A*). The curve is from data on a system containing five times the theoretical amount of ketone so as to force the equilibrium to the right in order to get conductivity curves more nearly approximating those of the sulphonic acid.

$$CH_3COCH_2CH_3 + H_2SO_3 \leftrightarrows CH_3C(OH)(SO_3H)CH_2CH_3$$

Conductivity-temperature curves were obtained at sulphonic acid dilutions of 120, 278 and 603 litres, each with five times excess ketone. Curves for the last two dilutions are shown in Fig. 3. The curves are of the butyraldehyde

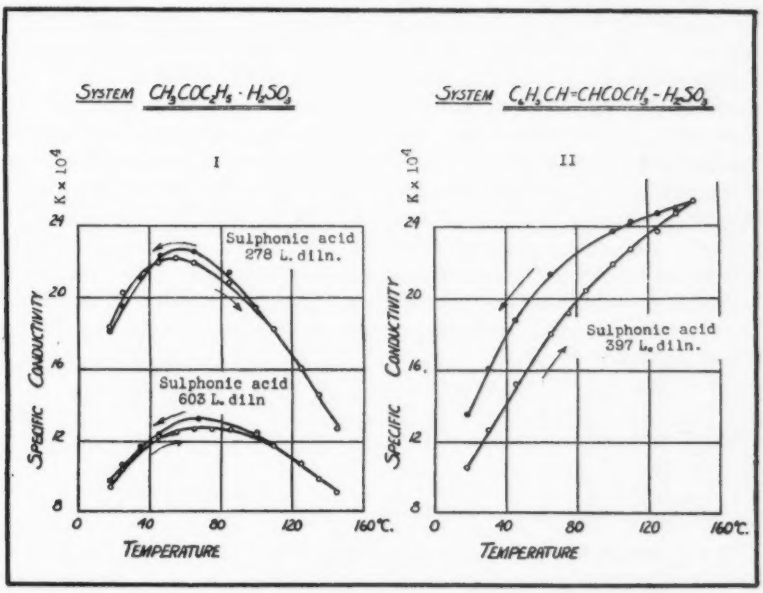


FIG. 3. *I. Specific conductivity of methyl ethyl ketone-sulphurous acid system.*
II. Specific conductivity of benzylidene acetone-sulphurous acid system.

type except that the maximum occurs at a lower temperature indicating earlier decomposition and that the down curves are more typical of sulphurous acid, showing that the recombination does not take place completely to give the former equilibrium on cooling the cell contents.

System:— Benzylidene Acetone—Sulphurous Acid

With this system equilibrium was only very slowly reached. In decimolar solution at 20° C. only 34% of the total sulphurous acid was combined after two days. The final equilibrium could however be approached by removing the excess sulphur dioxide in vacuum. Mixtures up to 83.7% sulphonic acid were obtained in this way without decomposition of sulphonic acid.

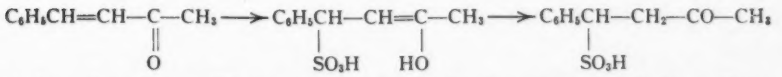
Conductivity-temperature curves were determined at sulphonic acid dilutions of 157 and 397 litres and showed a considerable and continuous rise with

increasing temperature over the whole range studied. The curve for the latter dilution is given in Fig. 3. The acid is therefore of the —C=C— type previously obtained for crotonaldehyde, *i.e.*, $C_6H_5CH(SO_3H)CH_2—CO—CH_3$.

If the sulphonic acid group were attached, initially, to the carbonyl group of the ketone, and were subsequently to change over at any temperature below the maximum of the usual curve for ketone sulphonic acids, *e.g.*, methyl ethyl ketone sulphonic acid, the fact would not be determinable from conductivity measurements alone. There is however considerable other evidence that the acid formed at 20° C. is much more stable than the sulphonic acid of a typical saturated ketone. In the first place the equilibrium of the latter is a very mobile one and is readily shifted on dilution and during iodometric determination of free sulphurous acid at room temperature. This is not the case with the sulphonic acid under discussion. Secondly, at the molarity of sulphonic acid corresponding to that obtained here the equilibrium is much further on the side of the sulphonic acid than for the corresponding compounds with saturated ketones (this acid, more than 83%; cyclohexanone sulphonic acid, approximately 60%; methyl ethyl ketone sulphonic acid, approximately 52%). The rate of hydrolysis for —C=C— acids is much slower than for —CHO acids. Typical curves for each of the crotonaldehyde sulphonic acids were obtained, as well as for saturated aldehyde sulphonic acids (benzaldehyde and vanillin), and these data, together with the observation of Hoover and Hunten that butyraldehyde sulphonic acid can be analytically determined from the sulphite produced by thirty minutes hydrolysis, are all in accord with this typical difference. The hydrolysis curves *E*, *F* and *G*, of Fig. 1, are for the acids $CH_3CH=CHCH(OH)SO_3H$; $CH_3CH(SO_3H)CH_2CHO$ and benzylidene acetone sulphonic acid respectively, each representing approximately the same sulphonic acid molarity. Curve *G* is of the ethylene linkage type. Since the acid in question is a ketone sulphonic acid it is of course more unstable than the corresponding crotonaldehyde compound.

The mechanism of this hydrolysis has been definitely established as involving a shift in the equilibrium through removal of free sulphurous acid as barium sulphite, since the results obtained by repeating the hydrolysis experiments using sodium hydroxide instead of barium hydroxide at the same normality showed no hydrolysis at 20° C. for reaction times up to four hours. Hoover and Hunten (27) had also previously observed that on attempting to hydrolyze butyraldehyde sulphonic acid with sodium hydroxide an equilibrium was set up for each concentration of the base.

It is not to be expected however that the actual mechanism consists of a direct addition to the double bond. As will be indicated later in the discussion of the reaction of sulphurous acid with certain unsaturated compounds, in which the ethylene linkage is the only active group, such direct addition takes place only slowly and at high temperatures. The present reaction proceeded at room temperature and hence it appears very probable that the addition takes place in the 1:4 position of the conjugated system followed by hydrogen migration according to the scheme:



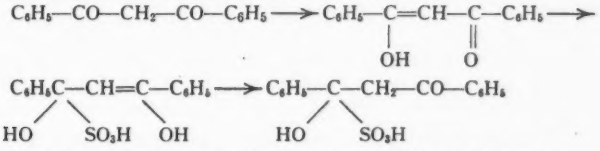
The fact that the equilibrium was attained slowly is in favor of this. Part of the delay in the establishment of the final conditions may have been due to the solid and insoluble character of the ketone, but the reaction mixture was kept well agitated and therefore, as in the case of glucose, the mechanism of the reaction would appear to involve the formation of an intermediate compound such as that postulated above, and whose concentration in the reaction mixture was always low.

System:— Dibenzyl Ketone—Sulphurous Acid

This ketone contains strongly negative groups attached to —CH_2—CO— groupings and therefore should exist to a considerable extent in the enolic form. It does not react to form a sulphonic acid. Thus on analysis the figures for free sulphurous acid (iodine titration) and total sulphurous acid (sodium hydroxide titration) gave the same value. The amount of sulphur in the aqueous and non-aqueous (ketone) layers was ascertained by micro-analytical determination. The figure for the aqueous layer corresponded within experimental error with that obtained volumetrically for free sulphurous acid, while in the non-aqueous layer, a trace of sulphur present was easily attributable to slight mixing with the aqueous layer. Further no reaction took place when the mixture was heated to 145° C. for two hours. The non-reactivity within this range of conditions is therefore definitely established.

System:— Dibenzoyl Methane—Sulphurous Acid

Dibenzoyl methane exhibits keto-enol tautomerism with either carbonyl group. It does not react with sulphurous acid. It might be expected to behave similarly to dibenzyl ketone, for, assuming enolization of one of the carbonyl groups, followed by 1:4 addition to the resulting conjugated system and hydrogen migration, there would result exactly the same compound as if addition took place to the ketone group directly, thus



The non-reactivity therefore indicates that 1:2 addition does not take place and further confirms the influence of negative groups attached to a —CH_2—CO— system.

Compounds Containing Cyclic Carbonyl Groups

In view of the possibility of formation of a carbonyl group by ketonization of a phenol nucleus in lignin, it was considered advisable to study the interaction of compounds containing cyclic carbonyl groups with sulphurous acid. Four such compounds were investigated, ranging from a cyclic ketone to certain phenols, namely, cyclohexanone, quinone, resorcinol and phloroglucinol.

System:— Cyclohexanone—Sulphurous Acid

The sulphonic acid of cyclohexanone, although still retaining the instability typical of ketone sulphonic acids, shows this property to a considerably smaller degree. Thus the equilibrium is further over on the side of the sulphonic acid than with methyl ethyl ketone and can be shifted to 95% sulphonic acid by use of two and one-half moles of ketone per mole of sulphurous acid. The equilibrium shift on dilution for the latter mixture is shown in Fig. 1, curve *D*. The greater stability over that of methyl ethyl ketone (curve *A*) at five times excess ketone is amply demonstrated. Due to the presence of this excess, the data are not directly comparable with those for benzaldehyde and vanillin, (curves *C* and *B*, Fig. 1) where equimolar reaction mixtures were employed.

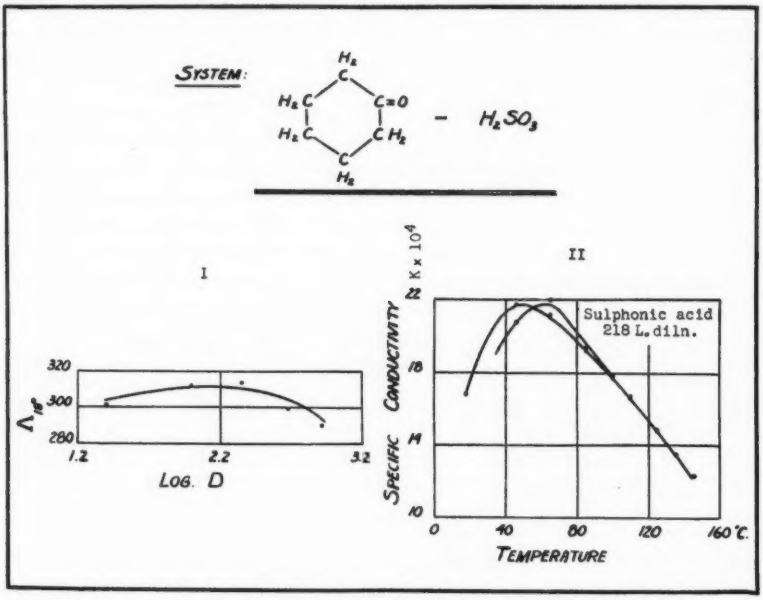


FIG. 4. *I. Equivalent conductivity of cyclohexanone sulphonic acid.*
II. Specific conductivity of cyclohexanone-sulphurous acid system.

In the actual order in which the work was experimentally carried out, this was the first sulphonic acid to show a comparatively mobile equilibrium and it was therefore considered necessary to determine whether the compound was a strong acid. The change in equivalent conductivity with dilution was studied at 18° C., the necessary free sulphurous acid corrections being obtained from the previous data of Hoover and Hunten (27). The resulting $\Lambda - \log D$ curve, Fig. 4, shows that the compound is a strong acid.

The change in specific conductivity with temperature is quite typical of saturated ketones, as shown in Fig. 4. Runs were made at dilutions of the order of 200 litres with 145° and 125° C. as respective maximum temperatures.

The data obtained indicated that the degree of permanent decomposition of the sulphonic acid at high temperatures depended on the extent and duration of the high temperature treatment, *i.e.*, with a lower maximum temperature or with a shorter time at high temperature (over about 110° C.), the original curve obtained during heating of the cell was more nearly repeated on cooling.

System:—Quinone—Sulphurous Acid

Quinone is rapidly and almost quantitatively reduced to hydroquinone with corresponding formation of sulphuric acid. A sulphonic acid is apparently not formed—in any event the possibility of its doing so is blocked by the rapidity of the other reaction.

System:—Resorcinol—Sulphurous Acid

A bisulphite derivative of resorcinol was prepared by Fuchs and Elsner (15). The reaction scheme postulated by them involved a primary conversion of resorcinol into its tautomeric alicyclic form, one mole of the latter then adding on three moles of the bisulphite.

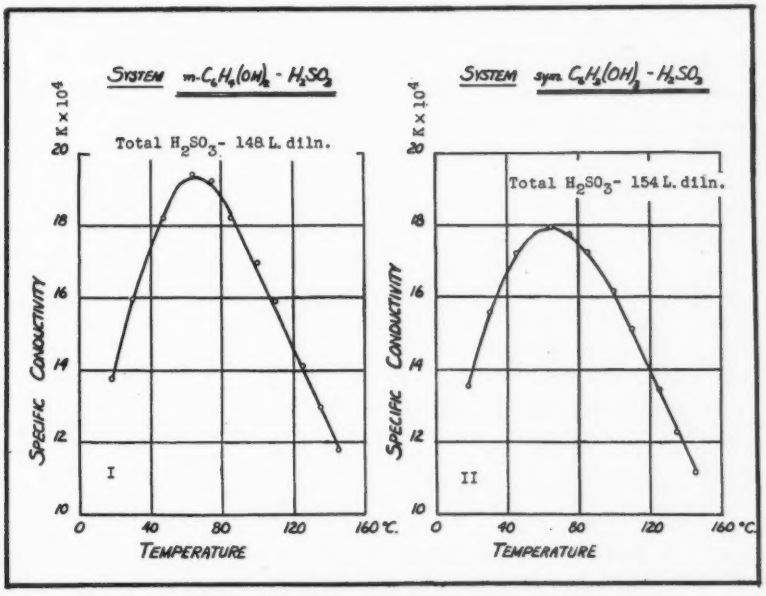


FIG. 5. *I. Specific conductivity of resorcinol-sulphurous acid system.*
II. Specific conductivity of phloroglucinol-sulphurous acid system.

Resorcinol, however, has been shown in the present investigation not to react with sulphurous acid between 18° and 145° C. No analytical combination was detectable at room temperature, and the conductivity curve (Fig. 5) is typical of sulphurous acid. The curve is strictly reproducible, the resorcinol preventing any oxidation of sulphurous acid. Any high temperature addition to an ethylene linkage formed by ketonization would be indicated on this curve.

This non-reactivity does not necessarily invalidate Fuchs and Elsner's results since in their experiments the more basic sodium bisulphite solution was employed. Even then their reaction was very slow indicating the small concentration of the keto form present at any time.

System:— Phloroglucinol—Sulphurous Acid

Fuchs (9) also investigated the reaction product of sodium bisulphite with ketonized phloroglucinol.

In the present study phloroglucinol was found to react very slightly with sulphurous acid to form the most unstable sulphonic acid yet encountered. The sulphonic acid was decomposed during iodometric titration of the free sulphurous acid even when an attempt was made to maintain the equilibrium in the presence of ice.

The conductivity-temperature curve is given in Fig. 5. The maximum is slightly less marked than that of the resorcinol curve indicating some combination. As in the case of resorcinol the curve obtained during cooling of the cell was identical with that for rising temperatures. The slight combination indicates phloroglucinol to be more easily ketonized than resorcin.

COMPOUNDS IN WHICH AN ETHYLENE LINKAGE IS THE ONLY REACTIVE GROUP

System:—Cinnamyl Alcohol—Sulphurous Acid

The interaction of cinnamyl alcohol and sodium bisulphite to form an unstable sodium sulphonate, $C_6H_5CH(SO_3Na)CH_2CH_2OH$, has been noted by Labbé (39). In this work it was found that if any reaction takes place between sulphurous acid and cinnamyl alcohol it does so very slowly even at 145° C. Analysis of the usual equimolar mixture showed no reaction in two days at room temperature. When heated in a sealed tube for 1 hr. at 145° C. the combined sulphurous acid amounted to 7.9% of the total, and in another sample heated for 6 hr. at 145° C., combination up to 15% took place. Although the tubes were flushed out thoroughly with nitrogen before loading it was practically impossible to prevent some air entering during sealing, so that even some of the observed combination may well be due to oxidation to cinnamic aldehyde by small amounts of enclosed oxygen, followed by combination of the aldehyde which would occur at once.

This was confirmed by conductivity measurements, which gave a sulphurous acid curve, Fig. 6. In the present instance, however, there is an objection to too strict a reliance on conductivity data. The solubility of the alcohol is very limited and as it was not possible to agitate the solution sufficiently during loading of the cell, nor to provide circulation in the cell itself, the liquid unquestionably contained considerable excess sulphurous acid. It is definitely established, however, that the presence of a carbonyl group in addition to an ethylene linkage greatly facilitates the addition of sulphurous acid to the latter and that therefore in the case of unsaturated aldehydes and ketones the —CO— sulphonic acid is probably an intermediate in the formation of the —C=C— acid.

System:— Tetrahydrobenzene—Sulphurous Acid

Tetrahydrobenzene has been shown to add on sulphurous acid at high

temperatures, giving a cyclohexane sulphonic acid.

After preliminary experiments had indicated that combination actually occurred, a number of sealed tubes, each containing 5 cc. of tetrahydrobenzene

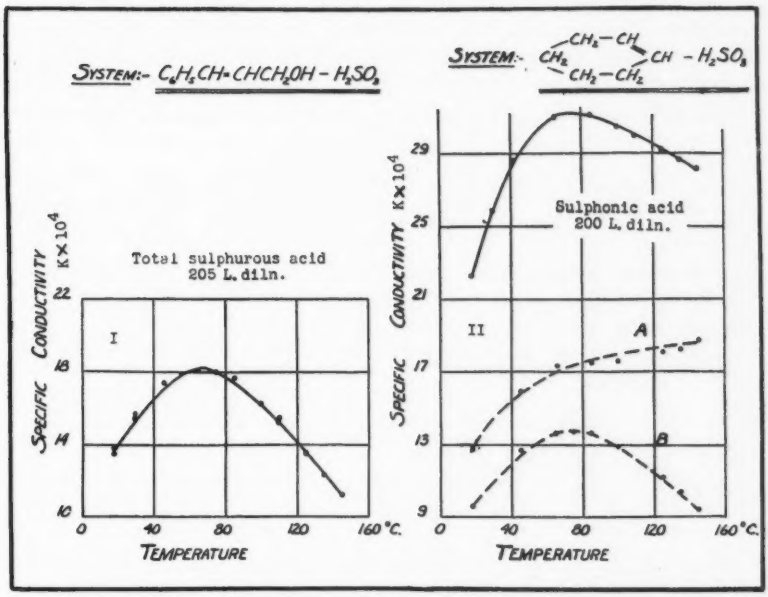


FIG. 6. *I. Specific conductivity of cinnamyl alcohol-sulphurous acid system. II. Specific conductivity of tetrahydrobenzene-sulphurous acid system. A. Specific conductivity of sulphonic acid (calc.). B. Specific conductivity of sulphurous acid. (Hoover and Hunten).*

and approximately 50 cc. of 0.1 *M* sulphurous acid were heated for one hour at 145° C. The tubes were flushed with nitrogen before loading and the heating time limited to one hour in order to obtain a solution for conductivity purposes as free as possible from sulphuric acid. The resulting liquid was poured into a nitrogen-filled flask and as much of the excess sulphurous acid as possible removed in vacuum. The mixture was then analyzed and a conductivity run made on a sample diluted to 200 litres total sulphurous acid. The data obtained are shown in Fig. 6 and are obviously the sum of two curves *A* and *B*, giving the conductivity of the sulphonic acid and that of the sulphurous acid present, respectively. It was possible to estimate the latter by interpolation between two curves for the conductivity of sulphurous acid obtained by Hoover and Hunten at two concentrations, one on either side of that shown by analysis to be present in the present instance, and hence to approximate to the true sulphonic acid curve by difference. The latter is seen to be the ethylene linkage type.

The rate of hydrolysis by barium hydroxide was also measured. Due to the extremely dilute character of the solutions used a considerable experimental

error was involved but the results indicated that no hydrolysis whatever took place as would be expected.

It is thus once again emphasized that sulphurous acid will only add on to an ethylene linkage very slowly and at high temperatures. The reaction with tetrahydrobenzene takes place much more readily than the corresponding reaction with cinnamyl alcohol (if indeed the latter takes place at all) but is still considerably more difficult to effect than when other groups are present which can react with sulphurous acid.

Furane Derivatives

The fact that α-furane derivatives show a certain degree of unsaturation and that the ring oxygen can form oxonium addition products has been recently emphasized by Moureu, Dufraisse and Johnson (41).

The interaction of furane derivatives and sulphurous acid was therefore considered as likely to be of interest and two such reactions were investigated; namely, those with furfuraldehyde and with furfuryl alcohol.

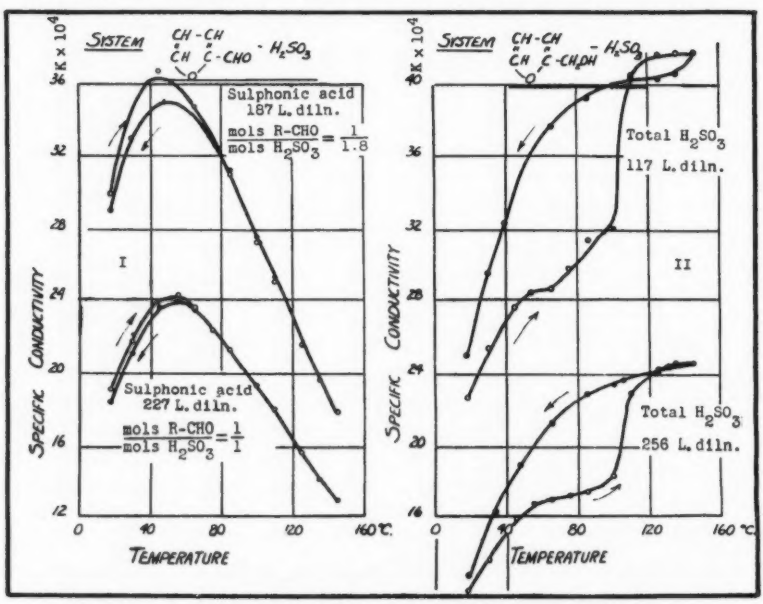


FIG. 7. *I. Specific conductivity of furfural-sulphurous acid system.*
II. Specific conductivity of furfuryl alcohol sulphurous acid system.

System:— Furfural—Sulphurous Acid

Furfural has been found to react like other nuclear aldehydes already studied (benzaldehyde and vanillin). The equilibrium of the usual reaction mixture was 88.4% on the side of the sulphonic acid (cf. benzaldehyde 92%, vanillin 80.7%). Furthermore the rate of hydrolysis with baryta was typically rapid, being complete in 10 min.

Conductivity-temperature curves, both for an equimolar mixture and with considerable excess sulphurous acid are shown in Fig. 7 and show that even in the presence of excess sulphurous acid no reaction with the furane ring takes place, and only the —CHO acid is formed.

System:— Furfuryl Alcohol—Sulphurous Acid

A gradual combination with sulphurous acid took place at room temperature. Chocolate-colored polymerization products were formed even in a nitrogen atmosphere. Conductivity-temperature curves, Fig. 7, were obtained the day after their respective reaction mixtures were prepared, and therefore before polymerization had set in to any extent, or the reaction had been completed. In both cases there was indicated definitely the formation of a sulphonic acid of the —C=C— type. This formation proceeds at a sufficient rate to show on the curves for temperatures above approximately 65° C. and appears to go extremely rapidly between 100 and 110° C. as indicated by the exceptionally rapid increase in conductivity over this range. On a second heating the down curve was reproduced.

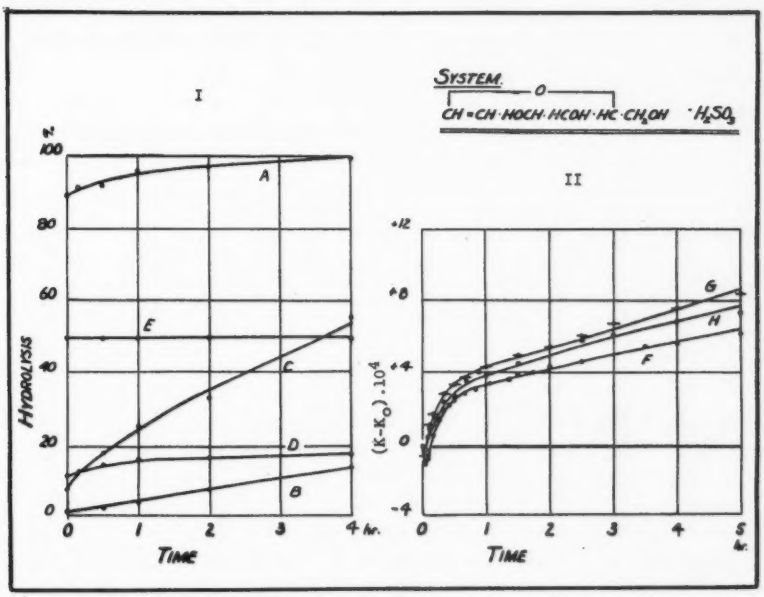
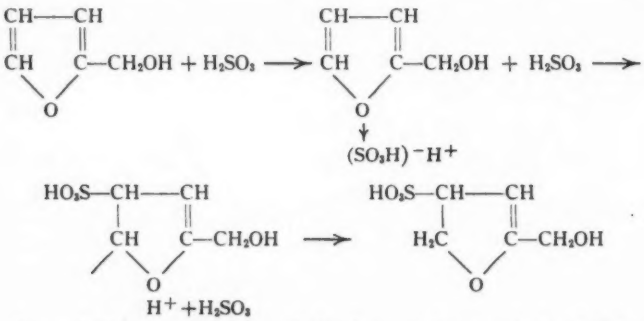


FIG. 8. *I. Hydrolysis of sulphonic acids with barium hydroxide. A. —CHO— acid of crotonaldehyde (0.0918 M). B. —C=C— acid of crotonaldehyde (0.0958 M) C. —C=C— acid of crotonaldehyde (0.0336 M). D. Furfuryl alcohol sulphonic acid (0.0678 M). E. Glucal sulphonic acid (0.0456 M). II. Rate of formation of —C=C— acid in glucal-sulphurous acid system. F. Temp. = 125° C. Cell heated rapidly. G. Temp. = 135° C. Cell heated rapidly. H. Temp. = 135° C. Cell held at 100° C. for ½ hr.*

Sealed tubes containing the reaction mixture were heated for 1 hr. at 110° C. to obtain the sulphonic acid for determination of the rate of hydrolysis with

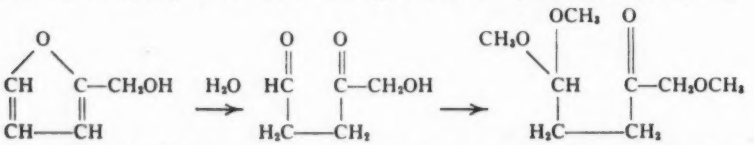
barium hydroxide. The resulting liquid was somewhat polymerized and on analysis showed that 82.3% of the total sulphurous acid was combined. The rate of hydrolysis with baryta is shown as curve *D* in Fig. 8. The sulphonic acid concentration was 0.0678 *M*. In the same figure, curve *A* gives the rate of hydrolysis for the —CHO reaction product of crotonaldehyde sulphonic acid [(CH_3—CH=CH—CH(OH)SO_3H)] at 0.0958 *M*, and curve *C* for the latter acid at 0.0336 *M*. On comparison with the other curves not only is the rate of hydrolysis seen to be very slow but the important point is at once brought out that practically all the hydrolysis that takes place, does so almost immediately. After the first few minutes the rate becomes almost negligible. Due to the insolubility of barium sulphite this cannot be due to the attainment of an equilibrium as would be the case if both reactants and reaction products were soluble but rather indicates that the sulphurous acid is combined in two different ways a small part being in unstable union and a larger percentage firmly bound.

Three possible mechanisms of reaction need to be considered in the light of these facts. The first involves the formation of an oxonium addition product, followed by entry of the sulphurous acid into the furane ring according to the scheme:



The second possibility is a scission of the furane ring, and the third a direct addition to the ethylene linkage.

In the second case, as shown by Pummerer and Gump (45), the first product would be δ-hydroxy-laevulinic aldehyde, CH_2OH—CO—CH_2—CH_2—CHO, since the dimethyl acetal of the latter was formed by treatment of furfuryl alcohol with methyl alcohol containing a trace of hydrochloric acid, thus



and yields of 40% laevulinic acid were obtained by heating furfuryl alcohol with 0.5% hydrochloric acid, or with 10% oxalic acid.

The experimental results, however, decidedly favor the first explanation. Thus it has been shown that the reaction takes place slowly at room tem-

perature, in approximately 0.1 *M* sulphurous acid. It hardly would be expected that scission of the ring would occur under these conditions. Secondly, the hydrolysis curve with baryta is typical of what would be expected if the first mechanism were true. Finally, the experimental data present a remarkable parallel with those observed for the pyrone derivative, glucal, which is discussed later and where conclusive evidence of the formation of an oxonium compound was obtained.

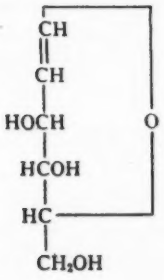
The fact that the product polymerizes very readily does not invalidate these conclusions since both furane and laevulinic aldehyde derivatives show this tendency to a marked degree. While the fact that furfuraldehyde does not show a similar reaction is indicative of some special mechanism like ring scission in the reaction of furfuryl alcohol with sulphurous acid, yet even this is discounted by the fact that furfural is one of the most stable of furane derivatives.

In view of the preceding work on unsaturated derivatives, direct addition to the ring is most unlikely. Even with tetrahydrobenzene such a reaction only takes place at high temperatures and furane derivatives show much greater saturation than this typically unsaturated compound.

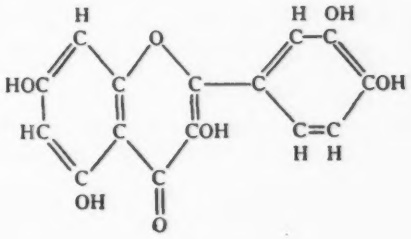
It is therefore concluded that, dependent on the other ring substituents, furane oxygen is capable of forming an oxonium addition product with sulphurous acid and that when such a compound is formed it can then serve as a medium for the introduction of the sulphonic acid group into the ring.

Pyrone Derivatives

The pyrone derivatives studied in the present investigation were crystalline glucal III and the flavone derivative, quercetin IV.



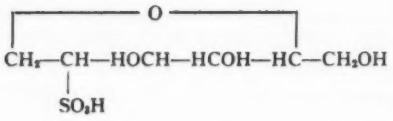
III



IV

System:— Glucal—Sulphurous Acid

Reaction took place slowly at room temperature. A conductivity-temperature curve was made on a mixture after two days standing (26% combined) at 204 litres total sulphurous acid dilution, Fig. 9. After passing through a sulphurous acid maximum, combination with the ethylene linkage was shown to take place at temperatures over 100° C. and this proceeded slowly and continuously, throughout the high temperature part of the cycle. Heating the



V

cell again gave the second curve indicating the formation of the —C=C— acid, presumably of structure V.

After five days standing at room temperature the sulphonic-acid content amounted to 56.7% of the total sulphurous acid and was raised to 75.4% by removing some excess sulphur dioxide in vacuum. Conductivity measurements, Fig. 9, showed that a comparatively unstable strong acid was present at low temperatures and was

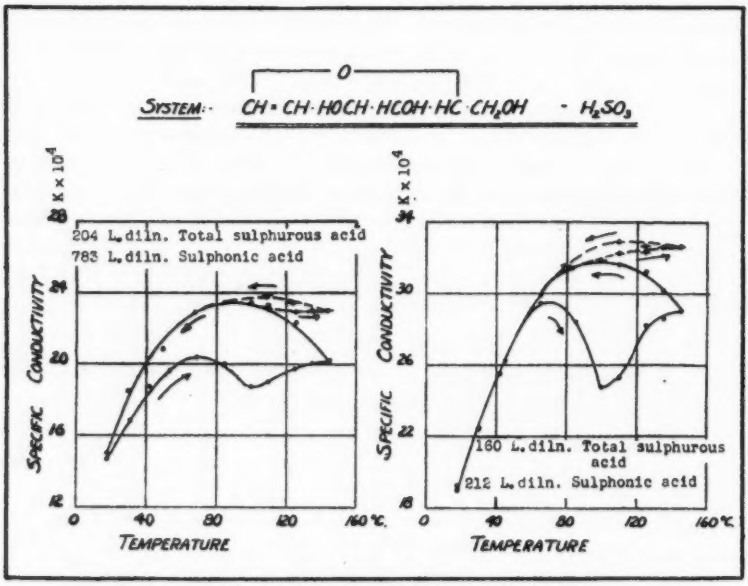
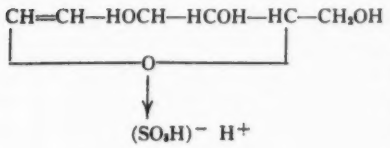


FIG. 9. *Specific conductivity of glucal-sulphurous acid system.*

decomposed on heating. At higher temperatures the —C=C— acid (V) was formed. The strength of the low temperature compound is shown by the fact that the specific conductivity at low temperatures was practically the same as that for the —C=C— acid. The decomposition is shown by the extreme character of the downward break above 65° C. On heating the cell a second time the —C=C— acid curve is obtained.

These facts, particularly the apparently high degree of dissociation and decomposition on heating, point strongly to the formation of an oxonium acid (VI) at low temperatures.

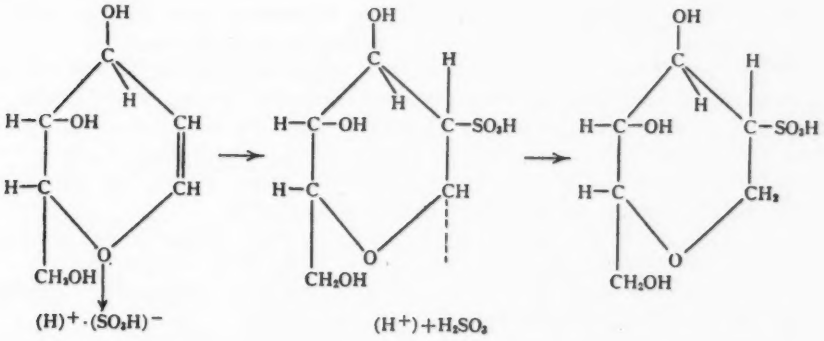
The data obtained when the reaction mixture was hydrolyzed with barium hydroxide are also in accord with these facts. Thus in the five-day reaction mixture (56.7% combined) 50% of the sulphonic acid was hydrolyzed imme-



VI

for furfuryl alcohol, namely,

diately and none thereafter for any period up to four hours. This indicated that the —C=C— acid (V) was also present at room temperature and hence was formed through the mechanism involving the oxonium compound according to the scheme previously discussed



The mixture, heated for three hours with barium hydroxide, in a sealed tube at 145° C., turned a brownish color. Analysis showed that 86.9% of the total sulphurous acid was combined, and of this 3% was immediately hydrolyzable, the remainder being unaffected for periods of hydrolysis up to four hours, thus confirming the high temperature formation of the —C=C— acid.

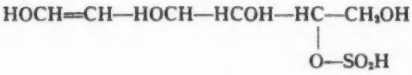
The change in specific conductivity with time was measured at critical temperatures, Fig. 8. For curve *F* the cell was heated rapidly to 125° C and held at that temperature. Curve *G* was obtained similarly at 135° C. For curve *H* the cell was heated to 100° C., held there for ½ hr. to permit decomposition of the low temperature reaction product and then heated to 135° C. (zero time). All three curves showed a slight fall in specific conductivity after reaching the final temperature indicating in every case incomplete decomposition of the oxonium compound when the final temperature was reached. The effect of holding the cell at 100° C. for ½ hr. is shown by the lower fall in conductivity in curve *H*. In spite of the fact, however, that curve *G* initially shows a lower (algebraic) change in conductivity with time, it shortly crosses curve *H*, which is for the same temperature, and eventually flattens out so that *G* and *H* become nearly parallel as shown in the figure. The only reason for any difference is that for curve *G* there was a higher concentration of the low temperature reaction product at zero time. Since the rate of formation of the —C=C— acid is increased by increased concentration of the supposed oxonium compound it would seem to be conclusively demonstrated that the latter is an intermediate in the reaction mechanism by which

the more stable compound is formed. Strong evidence in favor of a scheme such as that given previously is thus obtained.

Curve *F* at 125° C. shows a much greater initial fall in conductivity than either of the 135° C. curves. Although the zero time concentration of the supposed oxonium compound is greater (since the temperature is lower) the initial rate of formation of the —C=C— acid is slower than for curve *G*. It may thus be assumed that for equal concentrations of the oxonium compound the rate of formation of the more stable acid increases with rising temperature, which is quite in accord with expectations from the suggested mechanism. After the large initial drop in conductivity the reaction rate becomes nearly the same as that for curves *G* and *H* (the curves are nearly parallel) so that the loss due to lower temperature must be compensated for to a large extent by increased concentration of the oxonium compound. This is further evidence in favor of the proposed mechanism involving the consecutive reactions: glucal + sulphurous acid $\rightleftarrows$ oxonium derivative; oxonium derivative $\longrightarrow$ —C=C— sulphonic acid, since the equilibrium given by the first equation would be shifted to the left by increasing temperature.

A certain amount of direct addition to the ethylene linkage is also possible at the higher temperatures, analogous to that observed for tetrahydrobenzene. It would seem however that if direct addition does occur, the amount of stable acid so formed is relatively small up to about 135° C.

The only unstable reaction products other than oxonium derivatives would involve ring scission and contain sulphur linked through oxygen to carbon such as the compound VII. Any such products would be most unlikely. The stability of the pyrone ring in glucal is of course directly bound up with the ethylene linkage between the 1- and 2-carbon atoms and is of the same essential character as the pyrone ring in flavones and flavonols.



VII

The experimental facts are best explained by the assumption of the formation of an oxonium derivative of the type postulated and that the subsequent change to a more stable acid at a high temperature takes place through the mechanism suggested.

System:—Quercetin—Sulphurous Acid

Quercetin reacted extremely slowly due, at least in part, to its slight solubility. Even when excess sulphur dioxide was removed in vacuum the combined molarity could not be increased above 38% of the total theoretical amount since decomposition of the sulphonic acid resulted in further removal of sulphur dioxide.

A conductivity-temperature curve for a suitably diluted sample of this maximum combined liquid, Fig. 10, showed no formation of a —C=C— type acid at high temperatures. The probable reason for this is seen when a reaction scheme analogous to that for glucal is drawn up. The oxonium derivative

would be that indicated by (VIII) and the product formed by entry into the ring by (IX).

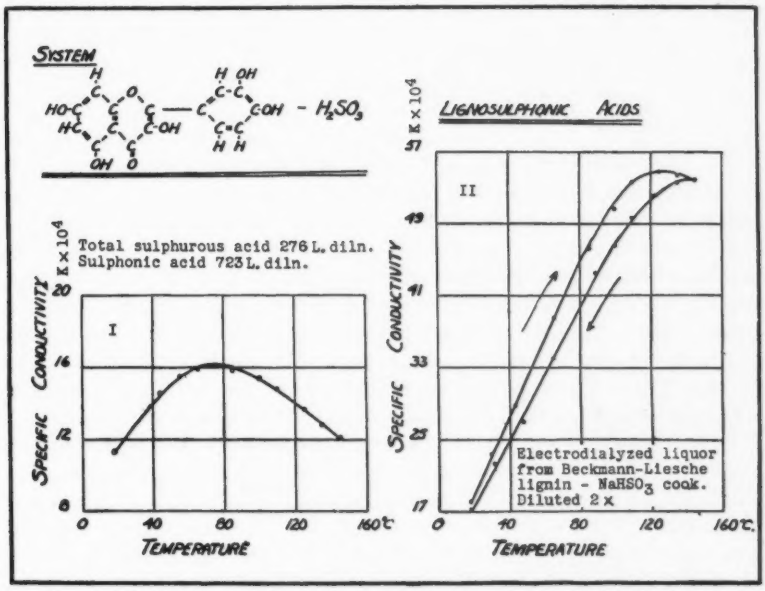
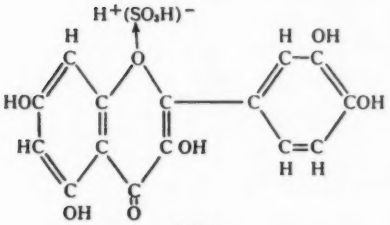
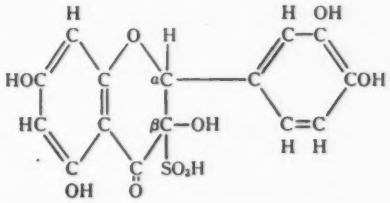


FIG. 10. *I. Specific conductivity of quercetin-sulphurous acid system. II. Specific conductivity of lignosulphonic acid from Beckmann-Liesche-Lehmann lignin.*

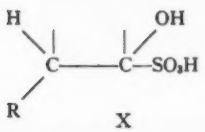


VIII



IX

The characteristic flavonol hydroxyl (on the β-carbon) makes the acid IX similar to that which would be formed by a cyclic ketone such as cyclohexanone since it contains the grouping (X); hence the acid should show ketone sulphonic acid characteristics and the conductivity curve which at first appears contradictory is thus brought into accord with the results previously obtained. This is further supported by the fact that hydrolysis with baryta is complete almost at once while the slow reaction rate and the final low concentration of sulphonic acid would be similarly explained by the theory.



X

The matter cannot be considered as definitely proved however until a derivative of the flavone type is investigated. If the latter reacts similarly to glucal the theory will be completely substantiated and an interesting essential difference between flavones and flavonols emphasized. In the meantime however the alternative possibility of slow hydrolysis of the flavonol derivative with the production of a keto hydroxy body must also be considered.

Lignosulphonic Acids

The main difficulty in studying the interaction of lignin with sulphurous acid is that the lignin isolated by any of the known methods is so changed during the process of its separation from the wood that it will not combine with free sulphurous acid even at high temperatures. Three sources of lignin were used in this investigation, namely, (a) the liquor produced by heating in a glass digester in a nitrogen atmosphere a mixture of sulphurous acid and spruce meal previously extracted with benzene-alcohol; (b) waste sulphite liquors obtained from the usual commercial processes of sulphite cooking; and (c) lignin isolated from straw by the Beckmann-Liesche-Lehmann method (1) and which was heated in sealed tubes with sodium bisulphite solution. The free sulphonic acids were in each case obtained by electrodialysis of the appropriate liquid.

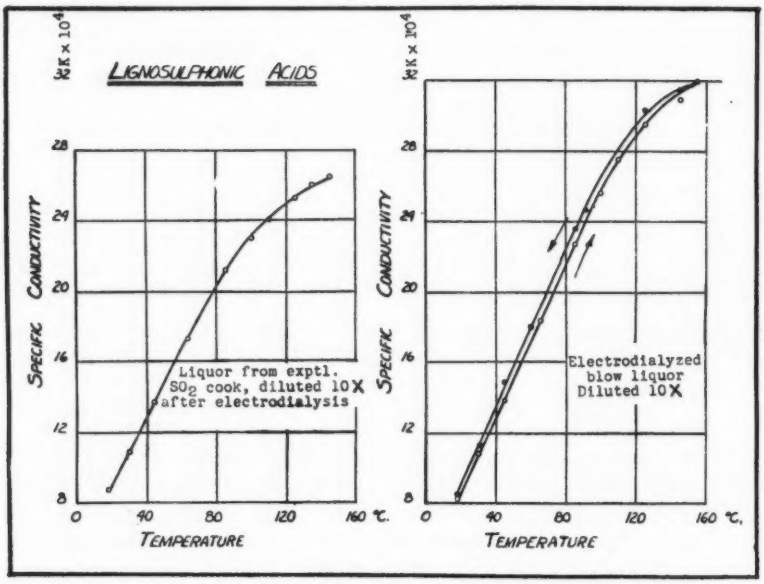


FIG. 11. *Specific conductivity of lignosulphonic acids.*

The products from the last two processes still contained a considerable amount of inorganic matter as shown by their ash content. In fact the electrodialysis of the waste liquors, although decreasing the quantity of ash,

brought about an increase in the ratio of ash to total solids. This is in accord with the results of other investigators (21), who found that the free acid always contained considerable amounts of mineral matter, and indicates something of the nature of inner complex salt formation by the high molecular complexes present.

Conductivity-temperature curves for the electrodialyzed liquors after suitable dilution are plotted in Fig. 10 and 11. It must be specifically emphasized that the absolute values of the conductivity mean very little and that the important point is the type of curve,—namely, one showing continually increasing conductivity with rise in temperature. It is thus established that in the final product the sulphonic acid group is attached to a —C=C— linkage of some sort. The curves give no indication as to the mechanism of formation, nor are any claims made as to the homogeneity of the products. The data submitted previously, however, indicate strongly that, besides an ethylene linkage either a carbonyl group, or a ring oxygen capable of oxonium salt formation, or both, play a role in the reaction. This point of view is supported by other evidence which points to the formation of an oxonium compound as an intermediate compound and which is discussed in the succeeding paragraphs.

Owing to the fact that some compounds present exert a buffer action it is not possible to determine accurately the total acidity of the sulphite liquors. For this reason the hydrolysis with barium hydroxide cannot be calculated as a "per cent hydrolysis" according to the usual method. Iodometric titrations can be run, however, and a comparison of the value obtained for iodine absorption with reaction time is obviously comparable with the usual baryta hydrolysis curves. Such data, obtained on the fresh sulphite liquor from the sulphurous acid reaction products showed some increase in sulphurous acid content immediately after treatment with barium hydroxide and none thereafter. The same liquors after standing for several days, and also the waste sulphite liquors, showed no increase whatever, a phenomenon somewhat similar to that observed with furfuryl alcohol and glucal.

This phenomenon, supposedly indicative of an unstable reaction product, has been noted by other workers. Thus Klason (30, 31, 36) concluded that sulphurous acid forms a stable chemical compound with lignin but that a certain amount of unstable compound is also produced. Samec and Rebek (47) showed that during dialysis, sulphite liquor loses sulphites which are "loosely bound" to the lignin and which they say are joined to carbonyl groups. Sulphite, bound as oxonium compound, would of course be even more easily removable than that attached to a carbonyl group. The latter would be decomposed only by removal of free sulphurous acid and consequent equilibrium shift. Furthermore, the general conception of the instability of carbonyl derivatives seems to be based on the erroneous notion that these compounds are sulphite esters and not sulphonic acids. This is shown by Fuchs' formulation of the reaction products of sodium bisulphite with the tautomeric alicyclic forms of resorcinol and phloroglucinol already mentioned.

When the reaction liquid obtained by heating Beckmann-Liesche-Lehmann lignin with sodium bisulphite was allowed to stand, or was electrodialyzed, a

precipitate was formed. This contained no sulphur and was not unlike the original lignin in appearance. Since it was formed in the presence of a marked excess of sulphite it may well have resulted from decomposition of an oxonium derivative. Its appearance in the middle compartment near the anode membrane of the electrodialysis apparatus suggests that it was not, like the precipitation on the anode in an outer compartment, of sufficiently small dimensions to pass through the parchment.

This mechanism of sulphonic acid formation would explain very reasonably the experimental conditions actually involved in the commercial manufacture of sulphite pulp. The fact that the digester must be held at 110° C. for some time before heating to a higher temperature would be a necessary deduction from a mechanism involving an oxonium derivative. Comparatively rapid combination with the lignin could only occur through the medium of oxonium addition. The addition product would be decomposed on heating to higher temperatures and the reaction therefore largely prevented. Also since the formation of the —C=C— acid in the case of glucal was shown to take place at a comparatively rapid rate only at 110° C. and higher, this is indicated as a highly probable critical temperature for a proper balance between the decomposition of the oxonium derivative and the formation of a —C=C— acid.

At this temperature direct addition to the ethylene linkage is also possible but should proceed very slowly. The fact that the formation of the —C=C— acid of crotonaldehyde ($CH_3CH(SO_3H)CH_2CHO$) is apparently autocatalytic (27) is of interest in this connection, since it is known that sulphite cooking is aided by the addition of spent liquor from a previous cook, and practically all the commercially used acid contains a certain amount of spent liquor.

In the authors' opinion therefore the formation of lignosulphonic acids probably takes place primarily through the medium of an oxonium addition product and to a very much lesser extent by direct addition to an ethylene linkage. The latter process proceeds very slowly but may become the main reaction during the later stages of the cooking. The former represents the mechanism by which the greater part of the lignosulphonic acid formation occurs and is particularly effective during the early stages.

The results of the present investigation also provide further and more convincing evidence for the existence of both a ring oxygen and an ethylene linkage in the same ring structure in the lignin molecule.

This investigation also shows that the suggested formation of the lignosulphonic acids by ketonization of a phenolic nucleus is most unlikely, and that the existence of a carbonyl group is not necessary for the formation of these acids or of the loosely bound sulphonic acids noted by other investigators.

Experimental Part

A detailed description of the apparatus used, the method of measuring specific conductivities, and the methods of analysis employed have been given in a previous communication (27).

System:— Crotonaldehyde—Sulphurous Acid

Crude crotonaldehyde was treated with anhydrous sodium carbonate to

TABLE I

HYDROLYSIS OF CROTONALDEHYDE SULPHONIC ACIDS

Agent	Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
				M/litre	%
$CH_3CH{=}CH{-}CH(OH)SO_3H$. Acid: total, 0.1022$M$; free, 0.0104$M$; combined, 0.0918$M$.					
$Ba(OH)_2$ sat'd. sol'n., 20cc.	At once	18.41	0.0921	0.0817	89.1
	10	18.79	0.0940	0.0836	91.2
	30	18.94	0.0947	0.0843	91.8
	60	19.68	0.0984	0.0880	95.8
	120	19.84	0.0992	0.0888	96.7
	240	20.21	0.1011	0.0907	98.8
$CH_3CH{=}CH{-}CH(OH)SO_3H$. Acid: total, 0.0371 M; free, 0.0042M; combined, 0.0329M.					
$Ba(OH)_2$ sat'd. sol'n., 20cc.	At once	6.16	0.0308	0.0266	80.9
	10	6.48	0.0324	0.0282	85.7
	30	6.80	0.0340	0.0298	90.7
	60	7.17	0.0358	0.0316	96.1
	120	7.33	0.0367	0.0325	98.8
	240	7.44	0.0372	0.0330	100
$CH_3CH(SO_3H)CH_2CHO$. Acid: total, 0.1012M; free, 0.0054M; combined, 0.0958M.					
$Ba(OH)_2$ sat'd. sol'n., 20cc.	At once	1.27	0.0064	0.0010	1.0
	30	1.44	0.0072	0.0018	1.9
	60	1.74	0.0087	0.0033	3.5
	120	2.61	0.0121	0.0067	7.0
	240	3.71	0.0186	0.0132	13.8
$CH_3CH(SO_3H)CH_2CHO$. Acid: total, 0.0339M; free, 0.0003M; combined, 0.0336M.					
$Ba(OH)_2$ sat'd. sol'n., 20cc.	At once	0.45	0.0028	0.0025	7.4
	10	0.92	0.0046	0.0043	12.8
	30	1.28	0.0064	0.0061	18.1
	60	1.77	0.0089	0.0086	25.6
	120	2.26	0.0113	0.0110	32.7
	240	3.78	0.0189	0.0186	55.3
$CH_3CH{=}CH{-}CH(OH)SO_3H$. Acid: total, 0.0312$M$; free, 0.0002$M$; combined, 0.0310$M$.					
NaOH	10	0.22	0.0011	0.0009	% decr.
	30	0.16	0.0008	0.0006	1-3
	60	0.10	0.0005	0.0003	constant
	120	0.16	0.0008	0.0006	within limits
	240	0.10	0.0005	0.0003	of exptl. error
$CH_3CH_2CH(SO_3H)CHO$. Acid: total, 0.0312 M; free, 0.0002M; combined, 0.0310M.					
NaOH	10	0.16	0.0008	0.0006	% decr.
	30	0.16	0.0008	0.0006	1-2
	60	0.16	0.0008	0.0006	constant
	120	0.10	0.0005	0.0003	within limits
	240	0.10	0.0005	0.0003	of exptl. error

NOTE:— *Temperature 20° C.; volume of liquid, 10 cc.*

neutralize crotonic acid and remove water, and then fractionally distilled. This process was repeated three or four times until a product of b.p. 103-104° C. was obtained. The aldehyde was used immediately after the final distillation.

The conductivity data for this system have been given (27).

The rate of hydrolysis with barium hydroxide of both the crotonaldehyde sulphonic acids was measured. The hydrolyses were carried out in 250-cc. ground glass stoppered bottles which had been thoroughly flushed out with nitrogen. In the usual procedure 10 cc. of the reaction mixture and 20 cc. of a saturated solution of barium hydroxide at 20° C. were allowed to react at 20° C. in a water thermostat for the required time, and the hydrolysis then stopped by rapid addition of a mixture of 20 cc. standard iodine (approx. $N/10$) and 10 cc. of 1:1 hydrochloric acid. The excess iodine was titrated with thiosulphate.

The —CHO acid (addition to aldehyde group) was obtained from the usual reaction mixture and the —C=C— acid (addition to —C=C— group) by heating the former in sealed tubes at 110° C. in an air furnace for one hour. The experiments were carried out for each acid at two different sulphonic acid molarities.

The mechanism of this hydrolysis was shown to be an equilibrium shift by repeating the hydrolysis experiments using sodium hydroxide instead of barium hydroxide and at the same normality and with the same experimental procedure. The results are given in Table I.

System:— Benzaldehyde—Sulphurous Acid

Kahlbaum's benzaldehyde was purified by washing with 1% sodium carbonate solution and then with distilled water (oxygen free), extracting with ether, drying over anhydrous sodium sulphate, decanting and distilling off the ether in a nitrogen atmosphere. Analysis of equimolar mixture:— Sulphurous acid: total, 0.1121 M; free, 0.0092 M; sulphonic acid, 0.1029 M (91.6% combined).

TABLE II

EFFECT OF DILUTION ON THE EQUILIBRIUM

Dilution calculated, litres	100	200	400	800
Dilution analyzed, litres	116	229	474	943

NOTE:— *Original reaction mixture; 0.1029 M sulphonic acid.*

TABLE III

RATE OF HYDROLYSIS OF BENZALDEHYDE SULPHONIC ACID WITH BARIUM HYDROXIDE

Time min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	21.98	0.1099	0.1010	97.8
60	22.38	0.1119	0.1030	99.8

NOTE:— *Sulphurous acid: total, 0.1121 M: free, 0.0089 M: sulphonic acid, 0.1032 M. Temp., 20° C.*

The sulphonic acid is decomposed with equilibrium shift on removal of free sulphurous acid. Analyses for the latter have to be carried out with ice in the analyzing mixture. The equilibrium is also shifted on dilution as is shown by Table II.

TABLE IV

SPECIFIC CONDUCTIVITY OF BENZALDEHYDE-SULPHUROUS ACID SYSTEM

Sulphonic acid 229 litres dilution				Sulphonic acid 227 litres dilution			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	185.4	145	144.4	18	180.8	110	190.2
30	220.0	135	159.4	30	218.5	100	207.5
45	246.5	125	175.6	45	246.5	85	235.5
65	251.8	110	202.1	55	251.8	65	268.3
75.6	243.5	100	219.9	65	281.8	58	272.2
85	231.3	85	248.1	85	230.9	45	266.5
100	209.9	65	278.5	100	205.3	30	231.0
110	194.2	45	289.8	110	190.2	18	200.5
125	171.4	30	255.3				
135	155.1	18	225.5				
145	144.4						

System:— Vanillin—Sulphurous Acid

Vanillin (Antoine Chiris, U.S.P. IX) was purified by recrystallization from aqueous alcohol, m.p. 80-81° C. After 24 hr. the equimolar mixture with sulphurous acid analyzed:— sulphurous acid: total 0.1120 *M*; free, 0.0214 *M*: sulphonic acid, 0.0906 *M* (or 80.7% combined). In the determination of free sulphurous acid, ice had to be used to freeze the equilibrium.

TABLE V

EFFECT OF DILUTION ON THE EQUILIBRIUM

Dilution calculated, litres	200	400
Dilution analyzed, litres	260	513

TABLE VI

HYDROLYSIS OF VANILLIN SULPHONIC ACID WITH BARIUM HYDROXIDE AT 20° C.

Time, min.	Cc. *N*/10 I_2 required	H_2SO_3 *M*/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	21.06	0.1053	0.0825	96.8
60	21.31	0.1066	0.0838	98.8

NOTE:— *Sulphurous acid; total, 0.1070M; free, 0.0228M: sulphonic acid, 0.0842 M.*

TABLE VII
SPECIFIC CONDUCTIVITY OF VANILLIN-SULPHUROUS ACID SYSTEM

R—SO_3H 260 litres diln. =64.0% total H_2SO_3		R—SO_3H 256 litres diln. =64.1% total H_2SO_3		R—SO_3H 523 litres diln. =59.2% total H_2SO_3	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	193.1	18	197.0	18	104.3
25	212.1	49.7	241.9	30	122.9
34.5	229.6	65	240.0	45	137.1
44.5	238.5	85	223.4	65	142.7
55.5	241.9	100	206.0	85	141.6
71.5	235.8	110	197.1	100	136.2
85	225.5	100	212.1	110	130.9
100	209.8	85	235.8	125	119.2
110	196.2	75	248.0	135	110.2
125	176.2	65	259.0	145	102.0
135	162.1	55	268.4	135	112.8
145	148.2	45	264.5	125	122.9
135	163.8	30	241.9	110	136.8
125	180.7	18	212.1	100	146.0
110	204.0			81	158.1
100	220.0			65	164.7
82	243.5			45	163.8
72	257.2			30	144.2
65	262.5			18	126.2
53	268.4				
41	268.4				
34.5	257.2				
25	240.1				
18	221.5				

System:— Glucose—Sulphurous Acid

Schering-Kahlbaum's "purest anhydrous" dextrose was used. The equimolar reaction mixture with sulphurous acid after standing for five days analyzed:— sulphurous acid: total, 0.0810 *M*; free, 0.0766 *M:* sulphonic acid, 0.0044 *M* (or 5.4% combined). A sample was transferred from the reaction vessel to a flask filled with nitrogen and excess sulphur dioxide removed with the suction pump. Analysis of resulting liquor:—sulphurous acid; total, 0.0215 *M;* free, 0.0183 *M:* sulphonic acid; 0.0032 *M* (or 14.9% combined). This was considered to at least approximate to the final equilibrium since some of the sulphonic acid had been decomposed.

TABLE VIII
SPECIFIC CONDUCTIVITY OF GLUCOSE-SULPHUROUS ACID SYSTEM

Temp. rising				Temp. falling			
Temp.°C.	$k.10^5$	Temp.°C.	$k.10^5$	Temp.°C.	$k.10^5$	Temp.°C.	$k.10^5$
18	452	100	393	145	255	65	487
43	514	110	359	135	283	46	497
55.5	524	125	312	125	313	30	460
65.8	495	135	283	100	398	18	399
85	444	145	255	85	446		

NOTE:— *Sulphonic acid = 312 litres dilution = 14.9% of total sulphurous acid present (i.e., sulphurous acid = 54.7 litres dilution).*

System:—Methyl Ethyl Ketone—Sulphurous Acid

Eastman's product was purified by distillation, b.p. 80-81° C. Analysis of an equimolar reaction mixture after 24 hr. standing:— sulphurous acid: total, 0.1575 *M*; free, 0.0878 *M:* sulphonic, 0.0697 (44.3% combined). After addition of more ketone (five times excess) analysis gave: sulphurous acid; total, 0.1575 *M*; free, 0.0307 *M*: sulphonic acid, 0.1268 *M* (80.5% combined).

TABLE IX

EFFECT OF DILUTION ON THE EQUILIBRIUM

Dilution calculated, litres	100	200	400
Dilution analyzed, litres	120	278	603

NOTE:—*Original reaction mixture—moles ketone: moles H_2SO_3 :: 5:1, with sulphonic acid, 0.1268 M.*

TABLE X

SPECIFIC CONDUCTIVITY OF METHYL ETHYL KETONE-SULPHUROUS ACID SYSTEM

R—SO_3H 120 litres diln. = 70.7% total H_2SO_3		R—SO_3H 278 litres diln. = 61.3% total H_2SO_3		R—SO_3H 603 litres diln. = 57.4% total H_2SO_3	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	362.1	18	183.8	18	93.2
25	381.8	25	203.4	25	103.8
35	395.4	35	223.0	35	115.3
45	389.5	44.5	219.8	45	122.2
55	375.3	55	222.1	54.5	125.3
65	358.2	65	219.8	65	127.7
85	320.3	85	209.0	85	127.2
100	285.0	100	194.6	100	124.2
110	262.5	110	182.3	110	117.9
125	229.8	125	160.8	125	106.9
135	207.8	135	146.8	135	98.2
145	185.7	145	127.9	145	90.6
125	229.8	135	147.8	135	98.4
100	285.0	125	160.0	125	107.1
65	360.5	85	212.6	100	125.0
44.5	387.5	65	225.8	67.5	135.8
35	378.9	46.5	223.9	45	125.0
25	360.5	34.5	211.6	35	116.8
18	343.9	24.5	194.8	25	106.2
		18	181.2	18	96.8

NOTE:— *Moles ketone: moles H_2SO_3 : : 5:1.*

System:— Benzylidene Acetone—Sulphurous Acid

Kahlbaum's product was recrystallized from benzene, m.p. 30-31° C. Equilibrium with sulphurous acid was only slowly attained. Analysis of usual equimolar mixture after three days standing gave:— sulphurous acid: total, 0.0642 *M*; free, 0.0389 *M*: sulphonic acid, 0.0253 *M* (39.4% combined). The mixture still contained undissolved ketone.

The following day excess sulphur dioxide was removed in vacuum, giving a liquid analyzing:— sulphurous acid: total, 0.0381 *M*; free, 0.0060 *M:* sulphonic acid, 0.0321 *M* (84.3% combined).

TABLE XI

SPECIFIC CONDUCTIVITY OF BENZYLIDENE ACETONE-SULPHUROUS ACID SYSTEM

R—SO_3H 154 litres diln. 79.2% of total H_2SO_3				R—SO_3H 397 litres diln. 83.7% of total H_2SO_3			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	259	145	553	18	105	145	255
30	304	135	547	30	127	134.5	250
48	369	123.1	544	45	153	125	248
65	417	110	533	65	180	110	243
75	440	100	524	75	192	100	238
85	465	83	500	85.1	205	65	214
100	493	65	462	100	219	45	188
110	507	44.5	406	110	228	30	161
125	537	30	361	125	238	18	136
135	544	18	308	135	248		
145	553			145	255		

TABLE XII

HYDROLYSIS OF BENZYLIDENE ACETONE SULPHONIC ACID WITH BARIUM HYDROXIDE AT 20° C.

Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	1.20	0.0060	0.0016	4.9
10	2.68	0.0134	0.0090	36.4
30	5.02	0.0251	0.0207	64.0
60	6.18	0.0309	0.0265	80.8
120	7.08	0.0354	0.0310	94.6
210	7.40	0.0370	0.0326	99.4

NOTE:— *Sulphurous acid: total, 0.0372 M; free, 0.0044 M: sulphonic acid, 0.0328M—volume of reaction mixture, 10 cc.*

System:—Dibenzyl Ketone—Sulphurous Acid

Eastman's "practical" grade was purified by washing with 1% sodium bisulphite, then with water, extracting with ether, drying over anhydrous sodium sulphate and recrystallizing from anhydrous ether; pale yellow needles, m.p. 29-30° C.

The product does not react with sulphurous acid under the experimental conditions studied. In equimolar mixture aqueous and non-aqueous layers result, both of which were analyzed. The micro sulphur analyses of the solutions were carried out with standard microanalytical apparatus according to the method of Pregl (44).

(1) Volumetric analysis of aqueous layer:—sulphonic acid: total, 0.0758 *M;* free, 0.0756 *M*. (2) Micro sulphur analysis of aqueous layer:— found: $BaSO_4$ per cc.; 17.38, 17.63 mg.; av., 17.50 mg. = 0.0750 *M*, *i.e.*, the same as the volumetric analysis for H_2SO_3. (3) Micro sulphur analysis of non-aqueous

layer:— found; $BaSO_4$ per cc.; 1.0, 1.3 mg., a quantity attributable to wetting by aqueous layer and indicating no combination. Dibenzyl ketone (2 gm.) heated with 40 cc. 0.1 *M* sulphurous acid in a sealed tube at 145° C. for 2 hr., after cooling, (aqueous layer) showed:— sulphurous acid: total, 0.0958 *M*; free, 0.0957 *M*, *i.e.*, no reaction over range studied.

System:— Dibenzoyl Methane—Sulphurous Acid

Dibenzoyl methane was purified by recrystallization from ligroin (60-70° C.); reddish-pink flat plates, m.p. 77-78°C.

The compound (1 gm.) was heated for two hours at 145°C. in a sealed tube (nitrogen atmosphere) with 40 cc. of approximately *M*/10 sulphurous acid. The aqueous layer on cooling analyzed:— sulphurous acid: total 0.0978 *M*; free, 0.0978 *M*, showing no combination.

System:— Cyclohexanone—Sulphurous Acid

Kahlbaum's product was purified by redistillation, b.p. 155.5-156.5° C. The equimolar mixture with sulphurous acid gave:— sulphurous acid: total, 0.1156 *M*; free, 0.0402 *M*: sulphonic acid, 0.0754 *M* (65.2% combined). More cyclohexanone was added to shift the equilibrium towards the side of the sulphonic acid. With 2½ moles of ketone per mole of sulphurous acid, analysis showed:— sulphurous acid: total: 0.1111 *M*; free, 0.0053 *M*: sulphonic acid, 0.1058 *M* (95.0% combined).

TABLE XIII

EFFECT OF DILUTION ON THE EQUILIBRIUM

Dilution calculated, litres	24.5	100	200	400	800
Dilution analyzed, litres	25.0	108	219	421	835

NOTE:— *Original reaction mixture; 0.1058 M sulphonic acid; moles ketone: moles* H_2SO_3 *: : 2.5:1.*

TABLE XIV

EQUIVALENT CONDUCTIVITY AT 18°C. OF CYCLOHEXANONE-SULPHONIC ACID

R—SO_3H diln. (anal.) litres	log *D*	H_2SO_3 *M*/litre (anal.) $\times 10^5$	k' (obsd.) $\times 10^6$	$k_{H_2SO_3}$ $\times 10^5$	k_{R-SO_3H} $(k'-k_{H_2SO_3})$ $\times 10^5$ calc.	Λ_{18} $(k.\phi)$
25	1.398	224.0	1276.0	70	1206	301.5
104	2.017	82.6	328.5	28	301	312
222	2.346	70.0	169.7	24	146	313
421	2.624	25.6	83.0	12	71	299
835	2.922	12.6	41.7	7	35	290

NOTE:— *Values for* $k_{H_2SO_3}$ *from Hoover and Hunten's data (27).*

System:— Quinone—Sulphurous Acid

Eastman Kodak's product was used. The usual equimolar mixture was prepared and the reaction shown by the following analyses to be one of reduction of quinone to hydroquinone with corresponding oxidation of sulphurous to sulphuric acid; (1) free sulphurous acid, 0.0043 *M*: total acid; (a) with brom

TABLE XV

SPECIFIC CONDUCTIVITY OF CYCLOHEXANONE-SULPHUROUS ACID SYSTEM

R—SO_3H = 219 litres dilution = 88% of total H_2SO_3				R—SO_3H = 222 litres dilution = 88% of total H_2SO_3			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
T °C.	$k.10^5$	T °C.	$k.10^5$	T °C.	$k.10^5$	T °C.	$k.10^5$
18	168.6	145	123.4	18	169.7	125	146.7
45.7	217.9	135	135.9	30	197.8	110	165.0
65	212.1	125	148.2	45	217.8	100	177.9
85	192.3	110	166.9	55	217.8	85	192.7
100	177.4	100	177.2	65	211.5	65	212.5
110	166.2	85	192.7	85	192.7	54	217.8
125	148.2	65	220.0	100	177.9	44.5	213.9
135	135.1	46	207.9	110	164.2	29.5	194.8
145	123.4			125	146.7	18	167.1

NOTE:— *Moles ketone: moles H_2SO_3 = 2.5:1.*

cresol green as indicator and calculating as the bisulphite end-point gave 0.1629 *M*—indicating other and much stronger acids than sulphurous to be present; (b) with phenolphthalein indicator, and calculating to the sulphate end-point gave 0.0829 *M*. Analysis of reaction mixture for sulphates: $BaSO_4$ per 25 cc. sample; 0.4274, 0.4258 gm., av., 0.4766: molarity of $BaSO_4$, 0.0820 *M*, *i.e.*, a check for the result for total acidity.

System:—Resorcinol—Sulphurous Acid

The usual molar mixture analyzed:—sulphurous acid: total, 0.1303 *M*; free, 0.1303 *M*.

TABLE XVI

SPECIFIC CONDUCTIVITY OF RESORCINOL-SULPHUROUS ACID SYSTEM

Temp. rising				Temp. falling			
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	137.9	100	169.8	145	117.7	63	192.4
30	159.6	110	159.0	135	128.1	45	172.8
47.2	182.0	125	141.0	125	141.0	31.2	160.2
64	194.1	135	129.8	110	159.0	18	137.9
74.5	192.2	145	117.7	100	168.0		
85	182.0			85	182.0		

NOTE:— *No sulphonic acid originally present; free sulphurous acid, 149 litres dilution.*

System:— Phloroglucinol—Sulphurous Acid

Kahlbaum's product "for analysis" was used. Some reaction takes place in equimolar mixture but the sulphonic acid formed is the most unstable yet encountered. It is decomposed during iodometric titration even when ice is used in an attempt to freeze the equilibrium. By varying the conditions of temperature, excess iodine, and speed of operation a maximum (for checked

results) of 7% of the total sulphurous acid (0.0940 *M*) was indicated as combined. In view of the instability of the reaction product this should represent a minimum for the true combination.

TABLE XVII

SPECIFIC CONDUCTIVITY OF PHLOROGLUCINOL-SULPHUROUS ACID SYSTEM

Temp. rising				Temp. falling			
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	135.4	100	161.8	145	111.8	65	180.7
30	155.8	110	151.2	135	112.2	43	172.1
44.8	172.1	125	134.5	125	134.0	30	155.8
64.4	179.4	135	122.7	110	151.2	18	135.4
74.5	177.3	145	111.8	100	162.2		
85	172.7			85	173.7		

NOTE:— *Total sulphurous acid, 154 litres dilution.*

System:— Cinnamyl Alcohol—Sulphurous Acid

Kahlbaum's cinnamyl alcohol was recrystallized from ether-ligroin; colorless needles, m.p. 32-33° C. In equimolar mixture with sulphurous acid only a small amount went into solution. After two days:—sulphurous acid: total, 0.0890 *M*; free, 0.0892 *M*, indicating no combination.

TABLE XVIII

SPECIFIC CONDUCTIVITY OF CINNAMYL ALCOHOL-SULPHUROUS ACID SYSTEM

Temp. rising				Temp. falling			
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	138.0	85	174.5	145	112.2	46	173.8
30	157.0	100	162.6	135	125.0	29.7	153.9
46	173.8	110	152.7	125	137.0	18	135.0
55.5	178.7	125	135.0	110	154.5		
65	180.2	135	123.8	85	174.5		
75	179.5	145	112.2	64	181.2		

NOTE:— *No sulphonic acid originally present; free sulphurous acid, 205 litres dilution. Due to the slight solubility of the alcohol considerable excess sulphurous acid was present.*

Two grams of the cinnamyl alcohol was heated in a sealed tube with 50 cc. of approximately 0.1 *M* sulphurous acid for 1 hr. at 145° C. After cooling the aqueous layer analyzed:— sulphurous acid: total, 0.0770 *M*; free, 0.0711 *M*: sulphonic acid, 0.0059 (7.9% combined). Another sample heated with more dilute acid for 6 hr. at 145° C. on cooling: —sulphurous acid: total, 0.0505 *M*; free, 0.0428 *M*: sulphonic acid, 0.0077 *M* (15% combined).

System:— Tetrahydrobenzene—Sulphurous Acid

Eastman's cyclohexene (5 cc.) was heated in a sealed tube with 50 cc. sulphurous acid for two hours at 145° C. After cooling the aqueous layer:—

sulphurous acid: total, 0.0895 M; free, 0.0634 M: sulphonic acid, 0.0261 M (29.2% combined).

Similar mixtures were heated for 1 hr. at 145° C., cooled, emptied into a nitrogen-filled flask and some of the excess sulphur dioxide removed in vacuum. The liquid analyzed:— sulphurous acid: total, 0.0418 M; free, 0.0157 M: sulphonic acid, 0.0261 M (62.4% combined).

TABLE XIX

SPECIFIC CONDUCTIVITY OF TETRAHYDROBENZENE-SULPHUROUS ACID SYSTEM

Temp. rising				Temp. falling	
Temp. °C.	$k.10^6$ observed	$k_{H_2SO_3} \times 10^6$ (Hoover & Hunten)	$k_{R-SO_3H} \times 10^6$ calc.	Temp. °C.	$k.10^6$ observed
18	223.3	95.8	127.5	145	282.0
30	258.5	—	—	135	287.5
42	286.2	127.5	158.7	125	292.0
65	315.1	136.4	173.7	110	298.5
85	311.3	136.7	174.6	100	306.5
100	305.0	129.3	175.7	85	311.3
110	300.0	—	—	67	311.3
125	292.0	112.0	180.6	44	286.2
135	286.2	103.9	182.3	30	255.2
145	282.0	94.4	187.6	18	221.9

NOTE:— *Free sulphurous acid originally in cell liquor, 0.00306 M/litre. Values for* $k_{H_2SO_3}$ *by interpolation between Hoover and Hunten's data at 0.00351 and 0.00276 M/litre.*

TABLE XX

HYDROLYSIS OF CYCLOHEXANE SULPHONIC ACID WITH BARIUM HYDROXIDE AT 20° C.

Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litre	%
60	3.22	0.0161	0.0004	0%
120	3.01	0.0151	none	within
240	3.14	0.0157	none	exptl. error

NOTE:— *Sulphurous acid: total, 0.0418 M; free, 0.0157 M; sulphonic acid, 0.0261. Volume of reaction mixture, 10 cc.*

System:—Furfural—Sulphurous Acid

"Refined" furfural from the Miner Laboratories, Chicago, was distilled under reduced pressure and redistilled immediately before being used, b.p. 55-56° C./17 mm.

The equimolar mixture gave:— sulphurous acid: total 0.0855 M; free 0.0103 M: sulphonic acid, 0.0752 M.

TABLE XXI

SPECIFIC CONDUCTIVITY OF FURFURAL-SULPHUROUS ACID SYSTEMS

$\frac{\text{mols R—CHO}}{\text{mols } H_2SO_3} = \frac{1}{1}$ R—SO_3H = 227 litres diln. = 76.5% of total H_2SO_3				$\frac{\text{mols R—CHO}}{\text{mols } H_2SO_3} = \frac{1}{1.8}$ R—SO_3H = 187 litres diln. = 53.5% of total H_2SO_3			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	188.5	145	129.2	18	300	145	178
30	218.5	135	142.1	44.5	367	135	195
42	239.0	125	157.8	65	348	125	218
56	241.5	110	179.8	85	310	110	253
65	235.0	80.5	219.8	100	273	100	276
75	224.3	65	235.0	110	250	85	312
85	214.1	45	235.8	125	217	65	348
100	193.3	30	211.8	135	196	48.5	351
110	179.5	18	183.9	145	178	30	330
125	157.8					18	291
135	142.1						
145	129.2						

TABLE XXII

HYDROLYSIS OF FURFURAL SULPHONIC ACID WITH BARIUM HYDROXIDE AT 20° C.

Time	Cc. *N*/10 I_2 required	H_2SO_3 *M*/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	16.22	0.0811	0.0710	94
10 min.	17.17	0.0859	0.0757	100

NOTE:— *Sulphurous acid: total, 0.0855 M; free, 0.0101 M; sulphonic acid, 0.0754 M.*

System:— Furfuryl Alcohol—Sulphurous Acid

Eastman's product was used. An equimolar mixture polymerized very readily, as shown by the development of a chocolate color about 48 hr. after mixing (nitrogen atmosphere). When samples were allowed to stand exposed to the air they turned dark brown in about half an hour. The progress of the reaction with sulphurous acid is shown by the analyses two hours after mixing: sulphurous acid: total, 0.0885 *M*; free 0.0881 *M*: sulphonic acid, 0.0004 *M*. Approx. 20 hr. after mixing:— sulphurous acid: total, 0.0840 *M*; free, 0.0743 *M*: sulphonic acid, 0.0097 *M* (11.5% combined). Two days after mixing:— sulphurous acid: total, 0.0795 *M*; free, 0.0604 *M*: sulphonic acid, 0.0191 *M* (24% combined).

Conductivity-temperature runs were made on each of two lots of reaction mixture the day after their preparation.

A quantity of the reaction mixture was heated in sealed tubes for one hour at 110 ° C. The resulting liquor was colored brown. On analysis it showed:—

sulphurous acid: total, 0.0825 *M*; free, 0.0247 *M*: sulphonic acid, 0.0678 *M* (82.3% combined).

TABLE XXIII

SPECIFIC CONDUCTIVITY OF FURFURYL ALCOHOL-SULPHUROUS ACID SYSTEM

Total H_2SO_3 117 litres dilution				Total H_2SO_3 256 litres dilution			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	226	145	417	18	120.1	145	246.2
30	255	135	405	30	138.4	135	245.2
44.5	276	125	403	45	158.2	125	242.0
54	284	110	403	55	166.8	105	236.5
65	286	100	399	65	169.9	100	234.1
75	298	85	393	75	172.1	85	228.5
86	315	65	377	85	174.4	65	211.9
100	321	39.5	324	100	194.2	47.5	190.3
110	405	30	298	110	228.5	33.6	162.3
125	416	18	251	125	243.5	18	129.9
135	417			135	246.2		
145	417			145	246.2		

TABLE XXIV

HYDROLYSIS OF FURFURYL ALCOHOL SULPHONIC ACID WITH BARIUM HYDROXIDE AT 20°C.

Time, min.	Cc. *N*/10 I_2 required	H_2SO_3 *M*/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	6.45	0.0323	0.0076	11.2
30	6.95	0.0348	0.0101	14.9
60	7.17	0.0359	0.0112	16.5
120	7.24	0.0362	0.0115	16.9
240	7.49	0.0375	0.0128	18.8

NOTE:— *Sulphurous acid: total, 0.0825 M; free, 0.0247 M; sulphonic acid, 0.0678 M.*

System:— Glucal—Sulphurous Acid

The product used was a carefully purified crystalline glucal. Reaction in the equimolar solution took place slowly as shown by the analyses. Two days after mixing:— sulphurous acid: total, 0.0890 *M*; free, 0.0659 *M*: sulphonic acid, 0.0231 *M* (26% combined). Five days after mixing:— sulphurous acid: total, 0.0805 *M*; free, 0.0349 *M*; sulphonic acid, 0.0456 *M*. Nine days after mixing:— sulphurous acid: total, 0.0717 *M*; free, 0.0155 *M*: sulphonic acid, 0.0562 *M*.

A conductivity-temperature run was made of the two-day reaction mixture. The results are given in Table XXV.

After removing excess sulphur dioxide from the five-day reaction mixture the liquid analyzed:— sulphurous acid: total, 0.0625 *M*; free, 0.0154 *M*: sulphonic acid, 0.0471 *M*. The liquid was diluted 10 times and taken through two cycles of temperature change in the conductivity cell. The results are given in Table XXVI.

TABLE XXV

SPECIFIC CONDUCTIVITY OF GLUCAL-SULPHUROUS ACID SYSTEM

First temperature cycle				Second temperature cycle			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	146.8	145	202.5	18	150.0	145	226.0
30.5	168.1	135	206.8	44.5	198.5	135	230.0
42	187.7	126	223.5	65	224.3	122	234.2
54	196.1	110	233.0	85	233.0	109	238.5
65	203.9	100	234.2	100	234.2	94.5	237.5
85	200.2	85	234.2	110	233.0	84.5	236.5
100	187.7	67.5	228.6	125	228.6	73	234.2
110	190.2	49.5	208.3	135	230.0		
125	197.2	30	184.9	145	226.0		
135	202.5	18	150.0				
145	202.5						

NOTE:— *Total sulphurous acid = 204 litres dilution = 24% of total sulphurous acid combined in the original reaction mixture.*

TABLE XXVI

SPECIFIC CONDUCTIVITY OF GLUCAL-SULPHUROUS ACID SYSTEM

First temperature cycle				Second temperature cycle			
Temp. rising		Temp. falling		Temp. rising		Temp. falling	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	192.2	145	290.5	18	190.1	145	326.0
30	224.2	134.8	302.1	47	262.1	135	326.0
42	255.2	125	312.5	65	295.0	125	326.0
65	294.0	110	316.5	85	313.8	110	329.0
85	284.0	83	315.0	100	319.0	79	315.0
100	248.0	64.8	295.0	110	323.0	64.5	297.5
110	253.5	45	262.1	125	323.0	45	258.6
125	282.0	30	224.2	135	326.0		
135	286.0	18	190.1	145	326.0		
145	290.5						

NOTE:— *Total sulphurous acid, 160 litres dilution. R—SO_3H, 212 litres dilution.*

The rate of hydrolysis with barium hydroxide of the five-day reaction mixture was determined with the results shown in Table XXVII.

A sample of the reaction mixture heated in a sealed tube at 125° C. for 3 hr. gave a brown liquid analyzing:— sulphurous acid: total, 0.0889 *M*; free, 0.0118 *M*: sulphonic acid, 0.0771 *M* (86.9% combined).

TABLE XXVII

HYDROLYSIS OF THE GLUCAL-SULPHONIC ACID REACTION MIXTURE WITH BARIUM HYDROXIDE AT 20° C.

Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	11.54	0.0577	0.0228	50.0
60	11.49	0.0575	0.0226	49.6
120	11.54	0.0577	0.0228	50.0
240	11.54	0.0577	0.0228	50.0

NOTE:— *Sulphurous acid: total, 0.0805 M; free, 0.0349 M: sulphonic acid, 0.0456 M.*

TABLE XXVIII

HYDROLYSIS OF A PREVIOUSLY HEATED SAMPLE OF THE GLUCAL-SULPHONIC ACID REACTION MIXTURE WITH BARIUM HYDROXIDE AT 20° C.

Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litre	%
At once	2.92	0.0146	0.0028	3.6
210	2.83	0.0142	0.0024	3.1

NOTE:— *Sulphurous acid: total, 0.0889 M; free, 0.0118 M: sulphonic acid, 0.0771 M. Volume of samples used, 10 cc.*

TABLE XXIX

VARIATION OF SPECIFIC CONDUCTIVITY OF GLUCAL—SULPHUROUS ACID AT 125° C.

Time hr.	Time min.	$k.10^5$	$(k-k_0)10^5$	Time hr.	Time min.	$k.10^5$	$(k-k_0)10^5$
0	0	194.4	—	1	0	227.2	+32.8
0	2	183.9	−10.5	1	16	229.3	34.9
0	4.5	186.8	− 7.6	1	30	233.0	38.6
0	10	200.3	+ 5.9	1	45	234.2	39.8
0	14.5	208.2	13.8	2	0	237.3	42.9
0	20	212.3	17.9	2	30	240.2	45.8
0	25	215.5	21.1	3	30	247.5	53.1
0	30	218.0	23.6	4	0	250.0	56.6
0	40	221.9	27.5	5	0	255.5	62.1
0	50	224.0	29.6				

NOTE:— *Total sulphurous acid, 203 litres dilution; sulphonic acid, 334 litres dilution. Conductivity cell heated rapidly to final temperature.*

System:— Quercetin—Sulphurous Acid

Eastman Kodak's product was used. Owing to the high molecular weight of quercetin and its limited solubility, a lower concentration of sulphurous acid than usual was used in the equimolar reaction mixture. The reaction proceeded very slowly and the equilibrium was far on the side of the original reactants, as shown by the analyses. Five days after mixing:— sulphurous acid: total, 0.0520 M; free, 0.0437 M: sulphonic acid, 0.0083 M (15.9% combined). Ten days after mixing:— sulphurous acid: total, 0.0513M; free, 0.0399 M: sulphonic acid, 0.0114 M (22.2% combined). Twelve days after

mixing (after removal of excess sulphur dioxide under reduced pressure): sulphurous acid: total, 0.0145 *M*; free, 0.0090 *M*: sulphonic acid, 0.0055*M* (33% combined). Since nearly half the sulphonic acid present had been decomposed, the final equilibrium-combined percentage was probably that indicated.

A sample of the liquid was diluted and a conductivity run made over the usual temperature range. The results are given in Table XXXII.

TABLE XXX

VARIATION OF SPECIFIC CONDUCTIVITY OF GLUCAL—SULPHUROUS ACID AT 135° C.

Time hr.	min.	$k.10^5$	$(k-k)_0 10^5$	Time hr.	min.	$k.10^5$	$(k-k_0)10^5$
0	0	173.9	—	1	1	216.7	+42.8
0	2	168.0	− 5.9	1	15	219.2	45.3
0	4	178.6	+ 4.7	1	30	223.4	49.5
0	6.5	184.9	11.0	1	45	226.0	52.1
0	10	190.2	16.3	2	0	228.4	54.5
0	20	201.5	27.6	2	31	234.2	60.3
0	30	206.0	32.1	3	0	242.0	67.9
0	41	209.7	35.8	4	0	250.0	75.9
0	51	213.0	39.1	5	0	257.0	82.9

NOTE:— *Total sulphurous acid, 216 litres dilution; sulphonic acid, 336 litres dilution. Conductivity cell heated rapidly to the final temperature.*

TABLE XXXI

VARIATION OF SPECIFIC CONDUCTIVITY OF GLUCAL—SULPHUROUS ACID AT 135° C.

Time hr.	min.	$k.10^5$	$(k-k_0)10^5$	Time hr.	min.	$k.10^5$	$(k-k_0)10^5$
0	0	186.5	—	0	45	222.0	+35.5
0	2	185.5	− 1.0	1	0	224.5	38.0
0	4	191.8	+ 5.2	1	30	231.5	45.0
0	6	196.4	9.9	2	30	245.2	58.7
0	10.5	202.0	15.5	3	0	248.5	62.0
0	20	208.1	21.6	4	0	255.3	68.8
0	30	213.8	26.3	5	0	260.5	74.0

NOTE:— *Total sulphurous acid, 208 litres dilution; sulphonic acid, 273 litres dilution. The conductivity cell was heated to 100° C., held at that temperature for half an hour and then the cell contents were raised to the final temperature (zero time).*

TABLE XXXII

SPECIFIC CONDUCTIVITY OF QUERCETIN—SULPHUROUS ACID SYSTEM

Temperature rising				Temperature falling			
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	113.8	100	153.8	145	121.2	60.9	157.0
44	146.0	110	148.2	125	137.0	42.2	146.0
54.5	155.0	125	136.9	110	147.0	18	113.0
65.5	158.9	135	128.4	100	153.2		
85	158.1	145	121.2	85	159.6		

NOTE:— *Total sulphurous acid, 276 litres dilution; sulphonic acid, 745 litres dilution.*

TABLE XXXIII

HYDROLYSIS OF QUERCETIN SULPHONIC ACID WITH BARIUM HYDROXIDE AT 20° C.

Time	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	R—SO_3H hydrolyzed	
			M/litres	%
At once	10.25	0.0513	0.0114	100
10 min.	10.25	0.0513	0.0114	100

NOTE:— *Sulphurous acid: total, 0.0513 M; free, 0.0399 M; sulphonic acid, 0.0114M.*

LIGNOSULPHONIC ACIDS

The first lignosulphonic acids studied were obtained by heating benzene-alcohol extracted spruce meal with sulphurous acid. After cooling, the liquor was filtered and then electrodialyzed in order to remove any sulphuric acid, excess sulphurous acid, and any other lower molecular complexes present. The apparatus employed was that of Pauli which has been described in detail elsewhere (42). Parchment membranes were used, and an auxiliary constant-head arrangement set up for the water supply to the outer compartments so as to limit pressure variations on the membranes. At the start of electrodialysis 110 volts were used and this value subsequently increased to 220 volts.

The liquor was electrodialyzed for 20 hr., after which it contained 0.052 gm. of total solids per cc. (dried in vacuum at 65° C.) and about 2 mg. of ash per cc. The liquor was diluted ten times and measurements made over the usual temperature range in the conductivity cell with results shown in Table XXXIV.

TABLE XXXIV

SPECIFIC CONDUCTIVITY OF LIGNOSULPHONIC ACIDS FROM SPRUCE MEAL AND SULPHUROUS ACID

Temperature rising				Temperature falling			
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	87.5	100	230.0	145	265.0	65	175.3
30	110.0	110	240.3	135	260.5	45	141.6
44	137.0	125	253.5	125	253.5	30	112.0
63.5	173.8	135	260.5	100	230.0	18	87.5
85	212.0	145	265.0	85	211.0		

TABLE XXXV

HYDROLYSIS AT 20° C. WITH BARIUM HYDROXIDE OF LIQUOR FROM SULPHUROUS ACID COOK

Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	Increase H_2SO_3 M/litre
At once	4.28	0.0214	0.0084
30	4.90	0.0245	0.0115
60	5.11	0.0256	0.0126
120	5.11	0.0256	0.0126
240	5.04	0.0252	0.0122

NOTE:— *Iodine titration of liquor equivalent to 0.0130 M H_2SO_3. Volume of samples used, 10 cc.*

Samples of waste sulphite liquors were obtained from the Howard Smith Paper Mills and from the Canada Power and Paper Co., and electrodialyzed. Conductivity data were obtained on the electrodialyzed liquor diluted ten times.

TABLE XXXVI

ANALYSES OF SULPHITE LIQUORS

	Howard Smith	Canada Power and Paper
	gm. per cc.	
Original liquors		
Total solids (65° C. in vacuum)	0.0977	0.0852
Ash	0.0108	0.0121
After 60 hr. electrodialysis		
Total solids (65° C. in vacuum)	0.0439	0.0491
Ash	0.0063	0.0096

TABLE XXXVII

SPECIFIC CONDUCTIVITY OF ELECTRODIALYZED WASTE SULPHITE LIQUOR

Liquor from Howard Smith Paper Mills				Liquor from Canada Power and Paper Co.			
Temperature rising		Temperature falling		Temperature rising		Temperature falling	
Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$	Temp. °C.	$k.10^5$
18	82.4	145	319.5	18	93.0	145	355.8
29.8	108.1	135	315.0	43	150.0	135	347.5
44.5	138.4	125.5	303.9	65	207.3	125	335.0
65	183.9	91	246.5	85	253.8	110	312.2
85	227.5	85	236.0	100	286.1	90	272.0
100	256.7	60	180.4	110	305.0	64.5	213.6
110	275.8	45.5	147.1	125	329.0	47.4	169.8
125	295.2	31	113.8	135	344.1	30	125.0
135	307.2	18	85.5	145	355.8	18	97.3
145	319.5						

The change in iodine titration on treatment with barium hydroxide (calculated as sulphurous acid) was measured in the case of the electrodialyzed Howard Smith waste liquor (Table XXXVIII).

TABLE XXXVIII

ATTEMPTED HYDROLYSIS OF ELECTRODIALYZED WASTE SULPHITE LIQUOR WITH BARIUM HYDROXIDE

Time, min.	Cc. $N/10$ I_2 required	H_2SO_3 M/litre	Increase H_2SO_3 M/litre
At once	1.43	0.0072	zero
30	1.53	0.0077	within
60	1.27	0.0064	exptl.
120	1.62	0.0081	error
240	1.57	0.0079	

NOTE:— *Iodometric titration equivalent to 0.0078 M H_2SO_3.*

Samples of lignin prepared by the Beckmann-Liesche-Lehmann (1) method were heated in sealed tubes with 3% sodium bisulphite solution in water free from oxygen. The tubes were flushed out with nitrogen before loading and heated at 110° C. for one hour and then at 125° C. for two hours. Some lignin went into solution. After cooling and electrodialyzing to remove excess sulphite the liquor contained: total solids, 0.0076 gm. per cc.; ash, 0.0020 gm. per cc. The liquid was diluted with its own volume of water free from oxygen and the conductivity determined (Table XXXIX).

TABLE XXXIX

SPECIFIC CONDUCTIVITY OF SULPHONIC ACIDS FROM BECKMANN-LIESCHE-LEHMANN LIGNIN

Temperature rising				Temperature falling			
Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$	Temp. °C.	$k.10^6$
18	181	100	506	145	537	88.5	436
30	234	110	536	135	536	65	339
43	288	125	548	122	521	48	270
6[illegible].8	387	135	544	110	496	31	224
85.5	464	145	537	100	466	18	171

Summary and Conclusions

1. The sulphonic acids of nuclear aldehydes are typically much more unstable than those with —SO_3H joined to aliphatically bound—CHO groups.

2. A quantitative method has been developed for following the equilibrium shift in solutions of these unstable sulphonic acids on dilution, and used to study their relative stability.

3. The reaction of sulphurous acid with compounds containing a latent aldehyde group such as glucose is characterized by a very slow velocity. A relation is indicated between the system glucose-sulphurous acid and the cyclic-open chain glucose equilibrium in aqueous solution.

4. The sulphonic acids of saturated ketones are very unstable. They are characterized by very ready decomposition and an equilibrium far on the side of the original reactants.

5. A method, based on some previous observations of Hoover and Hunten, for quantitatively measuring the rate of hydrolysis of the sulphonic acids with barium hydroxide, has been developed. The mechanism of this hydrolysis is a displacement of the aldehyde-sulphurous acid-sulphonic acid equilibrium. Its rate is a characteristic property of each type of sulphonic acid studied and has been used as a guide to the constitution of the sulphonic acids.

6. Unsaturated ketones form the —C=C— type sulphonic acids at room temperature. A mechanism for this reaction is proposed.

7. Compounds containing phenyl groups attached to a —CO—CH_2— linkage do not react with sulphurous acid over the temperature range 18-145° C.

8. Cyclic ketones react similarly to aliphatic ketones and give sulphonic acids and not sulphite esters.

9. For the system quinone-sulphurous acid the rapid oxidation-reduction reaction completely inhibits sulphonic acid formation.

10. Resorcinol does not react in its tautomeric alicyclic form with sulphurous acid over the temperature range 18-145° C.

11. A slight reaction takes place between phloroglucinol and sulphurous acid, but the sulphonic acid formed is extremely unstable.

12. The reaction of sulphurous acid with compounds in which an ethylene linkage is the only reactive group takes place only very slowly and at high temperatures. Confirmatory evidence is thus provided for the previously proposed mechanism of formation of —C=C— type sulphonic acids.

13. The reaction of furfural with sulphurous acid is that of a normal nuclear aldehyde.

14. Study of the system furfuryl alcohol-sulphurous acid shows that the sulphonic acid group enters the furane ring. This action takes place slowly at room temperature and very rapidly when the reaction mixture is heated. The preliminary formation of an oxonium addition product is indicated, and a mechanism for the complete reaction scheme for this system is proposed.

15. The interaction of the pyrone derivative, glucal, and sulphurous acid provides strong evidence for the primary formation of an oxonium addition product. At high temperatures the sulphurous acid adds on to the ethylene linkage of the pyrone ring. The rate of formation under critical conditions of this —C=C— type sulphonic acid shows definitely that the oxonium addition product is an intermediate in the formation of the —C=C— sulphonic acid. A mechanism for the reaction is postulated and strong confirmation found for a similar mechanism previously proposed for the reaction of furfuryl alcohol with sulphurous acid. Further confirmatory evidence based on the rate of hydrolysis of the sulphonic acids by barium hydroxide is submitted.

16. The reaction of sulphurous acid with the flavonol, quercetin, shows the same characteristics as that of other ketones. Possible reasons for this on the basis of the previously postulated reaction scheme are suggested.

17. Conductivity-temperature curves for lignosulphonic acids obtained from various sources indicate that the products are sulphonic acids of the —C=C— type.

18. Other evidence points to an oxonium addition product with a ring oxygen being a probable intermediate in the formation of the final and more stable lignosulphonic acid. The relation of such a mechanism to the experimental conditions employed in the commercial process of the manufacture of sulphite pulp is indicated.

19. The formation of lignosulphonic acids through the medium of a reaction involving a phenolic nucleus in its tautomeric form is highly improbable.

20. The fact is emphasized that for the formation of a lignosulphonic acid a carbonyl group does not necessarily have to be present.

21. Strong additional evidence is presented for the presence of a heterocyclic ring containing oxygen, and for an ethylene linkage in the lignin molecule. The latter is indicated to be in the same ring system as the oxygen atom.

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THE DISCONTINUITY IN THE VELOCITY COEFFICIENT OF A CHEMICAL REACTION AT THE CRITICAL TEMPERATURE[1]

By H. S. Sutherland[2] and O. Maass[3]

Abstract

An account is given of an hypothesis dealing with the mechanism of chemical reactions. This hypothesis was the *raison d'être* for the experimental work, which consisted in measuring the rate of a chemical reaction in a system over a temperature range including the critical temperature and under conditions such that there was a continuity of concentration, when passing from the liquid to the gaseous state of aggregation.

An experimental technique for the investigation of reaction mixtures under high pressures and at relatively high temperatures was developed. This method includes several new features which should find considerable application in investigations of this kind.

In the reaction investigated, *viz.*, that between propylene and hydrogen chloride, it was conclusively shown that the velocity of the reaction increases with rise in temperature in the liquid state, and that above the critical temperature the velocity of the reaction becomes practically zero. A new hypothesis was put forward with the object of suggesting further work. It depends on regional orientation of molecules in the liquid state which undergoes a rapid diminution at the critical temperature.

The work described in this paper was undertaken as a result of a definite hypothesis gradually developed in this laboratory during the last ten years, in connection with investigations of the reactions between halogen hydrides and unsaturated hydrocarbons. Briefly, this hypothesis is, that in certain reactions, the temperature coefficient is governed not solely by activation phenomena, but also by orientation of molecules, with the result that in these reactions it might actually have a negative value in a certain temperature range. With this in view, the investigation of the reaction between hydrogen chloride and propylene was undertaken over a temperature range, which extended well above the critical temperature, and under pressure conditions such that at high temperatures the density of the reaction mixture could be made to correspond to that prevailing in the liquid at the lower temperatures. Therefore, a brief review of previous work will be given, followed by a detailed account of the experimental data required.

The addition reactions between acetylene, allylene, ethylene, propylene, α-butylene, β-butylene, γ-butylene and the halogen hydrides in the liquid state were examined (4-12, 14) and it was shown that they fall into two classes. Acetylene and ethylene were found to be non-reactive, whereas the others reacted homogeneously and at a rate that could be reproduced with considerable accuracy. A striking experiment in this connection may be quoted (11, 12). A liquid mixture of 25 mole per cent propylene, 25% ethylene and 50% hydrogen bromide, when allowed to stand at room temperature in a sealed

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tube, was found on analysis to have undergone a reaction in which the propylene alone took part, while the ethylene and a corresponding amount of hydrogen bromide remained uncombined.

The question therefore arose early in these investigations as to the difference between ethylene and acetylene, and the other unsaturated hydrocarbons. It was thought that with the first two a decidedly smaller molecular attraction for the halogen hydride might account for their inertness. To test this, freezing-point curves were determined where experimentally possible, and it was indicated that whereas acetylene and ethylene do not form molecular compounds, all the others do. Consequently the first stage of the hypothesis mentioned in the opening paragraph may be summarized as follows. If a reaction is possible between *A* and *B*, the rate of this reaction is greatly increased if there is a molecular attraction between them, especially if this attraction is sufficiently great to induce molecular complex formation between *A* and *B*. In other words, the velocity of the reaction in the liquid state is influenced by the magnitude of the molecular attraction existing between the reactants. When two molecules, one of *A* and one of *B*, have a marked attraction for one another as molecules, it seems reasonable to suppose that there are chance approaches, where they will remain for a longer time in contact than if no such attraction existed. Furthermore this would be accompanied by an orientation conducive to interatomic change in the case when relatively large molecular attraction occurs. Where such a molecular attraction does not exist, a catalyst is necessary, and probably it functions in a manner to provide the equivalence of the molecular attraction in bringing the two together.

It is of course necessary for the reacting pair to have the proper activation in order to make the interatomic change possible. From this point of view a rise in temperature will increase the number of reacting molecules, *i.e.*, the rate of reaction, but on the other hand it will tend to diminish the number of properly orientated molecules, as well as the time of close approach due to increased thermal agitation. This will have a tendency to decrease the number of reacting molecules. This applies only to a system in which there exists a particularly pronounced attraction between the reacting pair. It is conceivable therefore that actually a negative temperature coefficient is possible in the case of a reaction where orientation and a tendency towards complex formation play a large part.

A number of experiments appeared to be in agreement with the views put forward above. Concentration of the reaction mixture should play an exceptional part in the rates of reaction. It was found that when propylene and hydrogen chloride in the gaseous state were mixed at atmospheric pressure, no reaction took place even after a year, although on the basis of concentration, with regard to any order of reaction, an easily detectable amount of combination should have occurred. Experiments followed in which the pressure of the gaseous reaction mixture was increased (9, 10). At higher pressures inconsistent results were obtained, due to the experimental difficulty in mixing the

constituents without at any time having a liquid phase appear. The moment a liquid phase appeared, reaction took place.

It is now possible to state more clearly the experimental problem which confronted the present writers. To carry out a thorough investigation, taking the reaction between propylene and hydrogen chloride as the most convenient to examine, a technique had to be devised to make possible the mixing of these substances in any desired proportions in the gaseous state. The mixture had then to be brought to any desired pressure above the critical temperature without at any time having the liquid phase appear. Furthermore it had to be possible to investigate the reaction temperature coefficient of the mixture at any temperature where the liquid might exist. To do this it was also necessary to determine the critical temperatures of the mixtures examined as well as the density of such mixtures, if any quantitative conclusions were to be drawn. The apparatus described has answered all of the above requirements and involves a number of new features which should prove useful in a large number of investigations carried out at relatively high pressures and temperatures.

Before proceeding to a description of the experimental work, a few more details concerning the propylene-hydrogen chloride reaction must be recapitulated. First, it has been definitely proved that the reaction in the liquid state is homogeneous and not influenced by the materials of the containers or by a mercury surface. Two separate investigations of this point will be published shortly. The product of the reaction between propylene and hydrogen chloride is mainly isopropyl chloride. A secondary reaction, which diminishes in amount with rise in temperature, also takes place (9, 10, 14). The only influence that this had in the present investigation was a slight correction which had to be made in connection with the analysis of the reaction at some of the relatively lower temperatures. It was shown that the velocity of the reaction greatly increases with the amount of hydrogen chloride present (9, 10). Consequently a mixture of two molecular volumes of hydrogen chloride to one of propylene was used throughout, because the rates of reaction of this mixture proved to be most convenient from the point of view of the time for an easily measurable amount of reaction to occur.

Experimental

Preparation of Reactants

The propylene was prepared by the dehydration of isopropyl alcohol over alumina at 360° C., and purified by low temperature fractionation, as described elsewhere (4, 5, 11, 12). The hydrogen chloride, prepared in the usual manner, was dried with phosphorus pentoxide and condensed with solid carbon dioxide in vacuum. It was redistilled and the middle fraction was stored for use.

Apparatus

The apparatus which had to answer the requirements mentioned in the introduction, passed through various stages of development, and several years of preliminary investigation were required. The final form, as developed

during the last year, in which all the difficulties were overcome, is the only one described. The early development was carried out by C. C. Coffin (5).

The reaction mixture, whether in the gaseous state during mixing, or later on when compressed to high pressure, was allowed to come into contact only with glass which had been carefully cleaned and mercury which had been subjected to careful purification and distillation. Moisture was eliminated by heating the apparatus while completely evacuated. The apparatus as

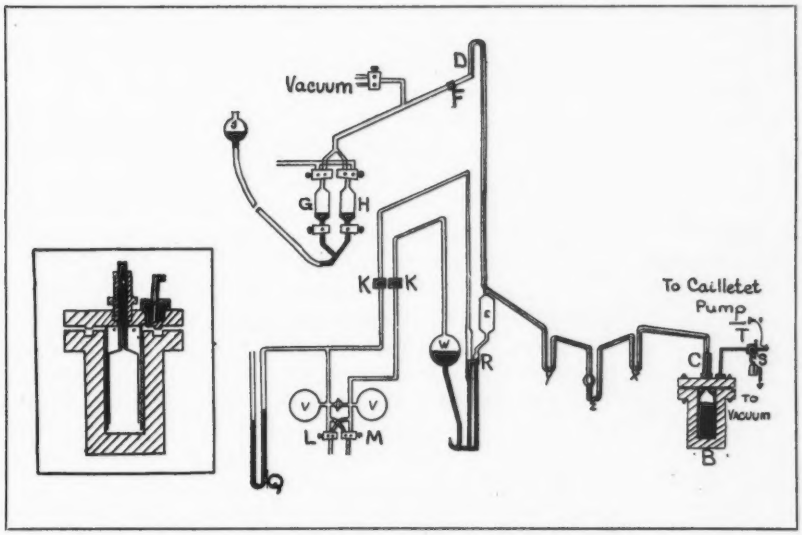

FIG. 1. *Diagrammatic representation of filling apparatus, reaction bulb and pressure apparatus.*

finally developed is shown diagrammatically in Fig. 1. A heavy thick-walled bomb, *B*, turned out of shafting steel was provided with a cover having two inlets. Into one of these, exactly in the centre of the cover, was threaded a large packing nut bearing a steel tube, *C*, which formed the sleeve of a metal joint fastened to the outside of the glass capillary. This will be described later. The glass tube, about 8 mm. Pyrex capillary, was passed through this sleeve and connected with a glass bell within the bomb, as shown in the figure. The capacity of the bell was about 100 cc., while the total capacity of the bomb was about half a litre. Into the inside of the bomb cover was threaded a piece of 2-in. iron pipe, as shown in the insert of the diagram. This was just larger than the glass bell, and served to protect it and eliminate any possible side sway, thus preventing the rigid capillary from breaking at the point where it joined the bell. In early experiments this was a major difficulty.

The bomb was about half filled with clean mercury, and the cover carrying the glass bell, capillary, metal joint, etc., was fastened on with six $\frac{1}{2}$-in. bolts. The bomb was then filled with oil (glycoline) through the other inlet, which

was connected by means of a steel capillary tube to a Cailletet oil pump and a pressure gauge. By pumping oil into the bomb the mercury could be forced up into the bell and act as a piston to compress the gases within it. In view of the relatively low critical pressures of the gases used, this method was preferred to the use of compressed gas for obtaining high pressures. Likewise the explosion risk was practically reduced to zero.

The Pyrex capillary connecting the bell-shaped compression chamber with the reaction bulb, *Z*, was bent in the form of a U, as was that connecting *Z* with the measuring bulb, *E*. The gas mixture was compressed by the Cailletet pump. This was a very good method for obtaining the high pressures but was useless for maintaining them for any appreciable time without continual pumping, owing to leaks in valves, etc. Hence a method was devised which gave the equivalence of an inexpensive absolutely leak-proof valve. Mercury could be frozen in *Y* while the gas mixture was in the compression chamber. The gases could then be compressed into the reaction bulb and mercury from the bomb forced over into the U-tube, *X*, where it was frozen. Thus the compressed gases could be kept under any conditions for any desired periods of time without the slightest possibility of either a leak or volume change. The remainder of the apparatus was used for mixing and measuring the reagents and will be described, along with the procedure for making a run.

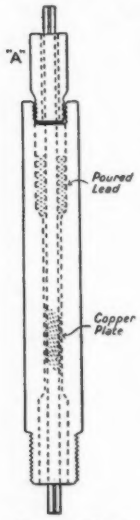

FIG. 2. *Metal sleeve attached to glass capillary with oil in pressure bomb.*

In the face of the flanged end of the bomb was a circular slot to hold a lead gasket, into which a corresponding ridge in the cover could be drawn by tightening the bolts. This gasket was protected from mercury by a layer of heatproof paint. Other gaskets were made of a phenol-resin-linen composition, such as is used for electrical insulation.

Metal Joint Attached to the Outside of the Glass Capillary

Essentially the problem called for a joint which would withstand more than 100 atm. pressure at 150° C. In general two main types of joints were investigated. First, those which would withstand oil pressure, and second, those which would hold mercury. The latter were the more difficult to make.

Fig. 2 shows the sweated type of joint. It consists of a steel sleeve about six inches long with a bore of 12 mm. The tube is constricted as shown in the diagram to about 8 mm.,—slightly larger than the size of the glass capillary tube. The cap, *A*, screws into the top of the sleeve, as shown, and has an opening just large enough for the capillary.

A three-foot length of Pyrex capillary was alternately swelled and constricted slightly about six inches from one end. This "corrugated" part of the tube was painted with a mixture of gold chloride in oil of lavender and allowed to dry at 100° C. By gradual heating, first in a smoky flame and then in a blast lamp, the oil was burned away and a thin film of conducting gold was fused into the glass. This film

was then electroplated about 1 mm. thick with copper and sweated into the constricted steel sleeve. The top part of the sleeve was filled with molten lead and the cap screwed on. The constrictions and swellings in the capillary prevented it from being forced out of the metal sheath by the pressure. The cap at the top stopped any side sway of the capillary and prevented it from snapping off at the top of the lead. In early types of joints the rigidity at this point caused such a difficulty.

A joint of this type is excellent for withstanding oil or gas pressure. Of course in contact with mercury the lead and copper parts were quickly attacked. Attempts were made to use various methods to protect them. Heat-resisting paints and high melting resins were poured in the bottom part of the metallic sleeve. These were used fairly successfully at room temperatures, but were non-permanent and permeable above 100° C.

Fig. 3 shows another type of joint, designed to withstand pressure when in contact with mercury. Its main advantage is its simplicity and the ease with which it may be taken apart. It consists of a flanged steel tube with rubber gasket and cover to fit. The tube is 3 in. long with a bore of 8 mm., and has a 2½-in. flange at one end. The steel cover, which contains a steel and a rubber disc, is fastened to the flange by four bolts as shown in the diagram. It is so arranged, that by tightening four bolts in the top of the cover any desired pressure can be exerted on the steel disc and thence on the rubber gasket. The cover and both discs have an 8-mm. hole in the centre. The Pyrex capillary was run through the tube, rubber gasket and steel disc. The cover was then bolted on, and the bolts in the top of the cover, X, were tightened. This forced the steel disc against the rubber and produced enough pressure to make an absolutely tight joint between the glass and the gasket Great care had to be taken to tighten all the bolts equally, so that no shearing could take place. The rubber was extruded somewhat along the capillary tube in both directions and tended actually to pull the glass apart. With capillary tubing below 6 mm., this presented a difficulty, but no trouble was experienced with the larger tubing or a tubing bulbed within the rubber gasket. This is the only type of joint with which any practical success was achieved in withstanding mercury pressures at temperatures above 50° C.

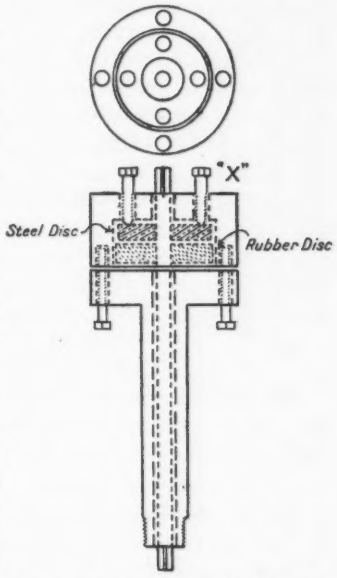

FIG. 3. *Metal sleeve attached to glass capillary with mercury in pressure bomb.*

Both types of joints were used in this investigation. In the earlier experiments a joint of the first type was used, it being only necessary to withstand

oil pressures. Later the apparatus was changed somewhat and the second type of joint was employed. This change consisted of merely inserting another bomb containing mercury between the oil pump and the bomb, *B*, Fig. 1. Thus only mercury was pumped into *B*. The advantages of this system are twofold. First, it eliminates any possibility of oil creeping around the bottom of the bell during manipulation, and second, it is not necessary to redistil the mercury when the bomb, *B*, is taken apart.

Glass Reaction Bulbs

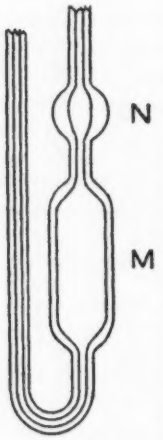

FIG. 4. *Reaction bulb.*

It was soon discovered that, although almost any thick-walled Pyrex bulb would withstand the pressure required, a certain design had a distinct advantage over all others. This advantage lay essentially in the technique of operation. Calculations showed that the reaction bulbs should have a capacity of from 0.2 to 0.3 cc. The disadvantage of such a small bulb was the difficulty in obtaining the correct mercury levels in the capillary tubing. A larger bulb corrected this, but the gases were compressed at one end of the bulb, giving a large mercury surface, an undesirable factor. Fig. 4 shows the type of bulb devised. It combined both the advantages of the small and large bulb. The upper section, *N*, had a capacity of about 0.25 cc., while the lower one held 3 cc. When the mercury to be levelled reached the small bulb, it dropped through to the bottom of the large bulb. The flow was then stopped by freezing the seal, *Y*, Fig. 1. The gases were then compressed and bubbled through this small quantity of mercury into the large bulb, and were finally compressed in the small bulb, *M* being by this time full of mercury. Thus the mercury surface in contact with the gas at high pressures is only twice the area of the capillary tube.

Manipulation of Apparatus

The mercury was first pumped up the capillary tubing, filling the reaction bulb, until it started to drop into *E*, Fig. 1. It was then frozen by surrounding *Y* with a carbon dioxide freezing mixture. The rest of the system was evacuated. The propylene and hydrogen chloride were admitted at known pressures to bulbs *G* and *H* respectively. Thus an equimolecular mixture, 2 : 1 mixture and so on could be obtained. By opening proper taps and raising the mercury reservoir, *J*, the gases were forced over into *E*. When the mercury had reached the mark, *D*, the taps at *G* and *H* were closed. The two mercury systems were thus kept entirely separate, so that no tap grease could be carried over into the measuring bulb, or reaction bulb. The bomb and manometer system of Pyrex were connected with the soft glass apparatus through a graded seal, *F*, and two deKhotinsky joints, *KK*.

By means of the two-way taps, *L*, *M*, pressure or vacuum could be applied to the mercury in the manometer connected to *E*. Thus it could be levelled at the zero mark, *R*, and the actual pressure of the mixed gases in *E* measured

on the open manometer, *Q*. The pressure and temperature having been measured, the mercury seal, *Y*, was melted and the gases were forced into the bomb by opening *S* to vacuum and applying pressure in the reservoir, *W*. As soon as the mercury started to drop through the reaction bulb the seal, *Y*, was frozen. Valve *S* was then closed and *T* opened, and the oil pump was operated. The gases were compressed slightly and the pressure released to get the small amount of mercury, which was in the bottom of the reaction bulb, back in the bomb. The pressure was then applied to the desired extent and the gases were compressed in the small reaction bulb. The mercury seal, *X*, was then frozen. With a little experience the amount of gas originally taken could be varied so that the compressed gases would just fill the small reaction bulb. The time taken to bring the gases from *E* into the small reaction bulb was about one minute. As soon as *X* was frozen, the oil pressure was released through the pump. At the end of the run the oil pressure was built up to about one third its original value and the seal, *X*, melted. Great care had to be taken at this point and the melting had to take place from the arms of the U down towards the bend. Otherwise the expansion of the mercury on melting cracked the glass tubing. The pressure was then released and the gases went back into the glass bell in the bomb. The seal, *Y*, was then melted and the gases were drawn back into *E*, after which *Y* was again frozen and the pressure and temperature of the gas read as before. Thus the amount of reaction was determined. The temperature in *E* could be regulated, in order that the reaction product (isopropyl chloride) would be measured as a gas. A typical example of the calculations involved, where reaction had taken place, illustrates the means of determining the extent of such a reaction.

Reactants measured in *G* and *H* (equal volumes): HCl, 250 mm.; propylene, 125 mm. Therefore the molecular proportions were:— propylene: HCl ::1: 2. Initial temperature of *E*, 27° C. Initial pressure of reactants in $E = 762 - 370 = 392$ mm. The gases were forced into the reaction bulb where they were condensed. Temperature of reaction bulb, 50° C.

After one hour. Temperature of *E*, 27° C. Pressure of products in *E*, 366 mm.

$$\% \text{ Reaction} = \frac{(\text{Initial-final})\text{pressure of reactants} \times 100}{\text{Initial partial pressure of propylene}}$$

$$\text{In this case} = \frac{(\text{Initial-final})\text{ pressure of reactants} \times 100}{\frac{1}{3}(\text{Initial pressure in } E)}$$

$$= \frac{3 \times (392 - 366) \times 100}{392}$$

$$= 19.8\%.$$

In carrying out true gas reactions the reactants always had to be kept above their critical temperatures. Hence the bomb, *B*, was heated in a large oil bath and the capillary tubing electrically. The temperature of the reaction bulb was controlled by a smaller transparent oil bath.

The pressures were measured on a hydraulic Bourdon gauge reading up to 3500 lb. per sq. in. This gauge was checked against the vapor pressure of

hydrogen chloride and was found to be accurate to about 3% at 1000 lb. and the necessary corrections were made over the entire scale.

When measuring the velocity of chemical reaction, the samples were subjected to the conditions of the experiment for a definite time, and then the amount of reaction determined by change in pressure in the measuring volume, *E*. The same sample was again compressed and left under the same conditions for a longer time and so on. In the case of liquid reactions the temperature of the reacting mixture was kept below the critical temperature of the mixture. The formation of secondary product and its subsequent partial entrainment in the mercury made the low temperature liquid runs less accurate than similar determinations by other methods (direct analysis).

The densities of the gas mixtures were determined very simply as follows. A definite amount of gas mixture, measured by pressure in known volumes, was compressed into the small reaction bulb, the volume of which was previously determined. Hence after one or two experiments it was possible to know approximately how much gas to add, to fill the bulb exactly at definite pressures, and points on the density curve could be obtained. For some of the values the bulb was only partially filled with gas and estimations of volume were made. Points determined in this way agreed fairly well with those obtained by completely filling the bulb.

Since the pressure could be controlled at will to a value far above the critical pressure of either component, it was a simple matter to determine the critical temperature of any mixture. The desired mixture was compressed into the reaction chamber and the temperature lowered until the meniscus appeared, the pressure being kept above the critical pressure. Since, as shown later on, no reaction between propylene and hydrogen chloride occurs above the critical temperature of the mixture this temperature could be determined accurately.

Experimental Results

Since propylene and hydrogen chloride have been shown to react in the liquid state and to be unreactive as gases under ordinary conditions, the study of gas reactions under high pressures offered two possible results. On the one hand, there might be no reaction under the highest pressure possible with the apparatus, providing the reactants were still gases. This would point to the fact that the proximity of the molecules to one another has little or nothing to do with the phenomenon observed. On the other hand there might be a reaction threshold at a definite pressure, in which case the distances between the molecules would have a great effect.

Previous experiments at high pressures had given divergent results. Occasionally a reaction mixture could be obtained at high pressures, which even after several hours showed no reaction whatever. It was impossible however to obtain this result repeatedly. Since there always was the possibility of the presence of the liquid phase due to adsorption on impurities in the reaction chamber, the negative results, as far as reaction velocity is concerned, are the only ones that really count. Nevertheless the uncertainty which would always exist made it imperative that further experimentation

be undertaken. It was only with the apparatus as finally described, and then only when particular care was taken in the purification of the mercury, and the cleaning of the glass, that the results, whatever they were, could be taken as having a definite meaning.

The first experiments in this category were carried out at 105° C. under pressures up to 100 atm., using both 1:1 and 1:2 proportions of propylene and hydrogen chloride. The results are given in Table I.

TABLE I

REACTION BETWEEN PROPYLENE AND HYDROGEN CHLORIDE IN GASEOUS PHASE

Experiment No.	Temperature °C.	Pressure atm.	Mixture (HCl: Propylene)	Time hr.	Result
1	105	26	1:1	2	No reaction
2	105	26	2:1	5	No reaction
3	105	34	1:1	2	No reaction
4	105	34	2:1	2	No reaction
5	105	50	1:1	2	No reaction
6	105	50	2:1	2	No reaction
7	105	83	2:1	2	No reaction
8	105	100	2:1	2⅔	No reaction
9	105	105	2:1	4	No reaction
10	105	105	2:1	10½	No reaction

When it is remembered that at 0° C. the reaction proceeds to 17.5% and at 50° C. to 40%, for a 2:1 mixture in 2 hr., one can draw the definite conclusion that relative to the velocity of reaction in the liquid state the velocity is 0 at 105° C. up to pressures of 105 atm. Experiment 10, in which the reaction mixture was kept for 10½ hr., is most convincing, since in the liquid state at a much lower temperature it would have gone to completion in that time.

The idea might occur that somehow or other the reaction mixture might have become unreactive. To prove that this was not so, the following procedure was followed. A definite mixture was placed in the reaction chamber above the critical temperature, kept there for a time, then returned to the measuring apparatus and found to have remained uncombined. This same mixture was then compressed under critical conditions and the temperature of the bath around the reaction bulb lowered to below 50° C. After an adequate time the reaction mixture was again brought into the measuring apparatus and in every case it was found that at the lower temperature, where the liquid state existed, reaction had taken place. For instance, a 2:1 mixture was kept at 105° C. under 100 atm. for 4 hr., no reaction taking place, but, when subjected to a temperature of 45° C. for 65 min., 20% was found to have reacted. Another 2:1 mixture was kept for 2 hr. at 105° C. under 100 atm., no reaction resulting. This same mixture was brought back to the reaction chamber at 105° C. and 100 atm. and then subjected to a lower temperature (35° C.) for a shorter time and was found to have reacted to the extent of 19%. These are instances of a large number of experiments, all of which gave similar results.

It is obvious that in discussing these results the density of the reaction mixture is of interest both in the gaseous and liquid states. The methods of determining these densities and the critical temperatures of the mixtures have already been described. The following data were obtained.

TABLE II

CRITICAL TEMPERATURES OF PROPYLENE-HYDROGEN CHLORIDE MIXTURES

Propylene, mole %	100	66.6	50*	33.3	0
Critical temperature, °C.	92.1	84	75	70	52

Burst the bulb on appearance of less dense liquid phase.

TABLE III

DENSITIES OF A MIXTURE OF PROPYLENE AND HYDROGEN CHLORIDE (1:2)

	Liquid			Gas					
Temperature, °C.	0	20	50	78	78	78	78	78	78
Pressure, atm.				61	71	74.5	76.5	80.5	102
Density, gm. per cc.	0.80	0.73	0.40	0.16	0.20	0.22	0.24	0.26	0.42

The relation between critical temperature and composition (Table II) is quite typical of a binary mixture in which one component is more volatile than the other. In such a mixture the gaseous phase even considerably above the critical pressure is never absent above the critical temperature of the more volatile compound (in this case, the hydrogen chloride). Hence 52° C. is the highest temperature at which an all liquid phase is possible at pressures up to 100 atm. The 2:1 mixture has a critical temperature of 70° C., so that above this temperature only the gaseous phase can exist. Between 52 and 70° C. for a 2:1 mixture there will therefore always be present a certain amount of gaseous phase, and liquid phase, the relative proportions of which diminish with rise in temperature.

Table III shows that the density of the 2:1 gas mixture above its critical temperature and at a pressure of 100 atm. is greater than the extrapolated density of the liquid mixture for that temperature, and is actually equal to the density of the liquid mixture at 50° C.

A few words to recapitulate what is known up to this stage are now in order. The velocity of reaction of a 2:1 mixture in the liquid state was measured by Sivertz (9, 10) at 0 and 20° C. and was shown to have a decidedly positive temperature coefficient. It is known therefore that up to 20° C. the velocity of the reaction increases, but that at 105° C. it is immeasurably small. Furthermore, that this is not a question of concentration is shown by the density measurements (Table III). When these results were obtained the authors speculated as to the form which the rate of reaction-temperature curve would take. That an inversion takes place somewhere between 20 and 105° C. is obvious.

Two questions therefore arise. First, will a reaction take place at all above the critical temperature? Second, does the temperature coefficient of the velocity of reaction in the liquid state show a point of inversion before the critical temperature is reached? Attempts to answer these two questions will be made in the two subsequent sections.

Experiments were made above the critical temperature and in the range where they were conclusive. The data are shown in Table IV.

TABLE IV

RESULTS OBTAINED IN EXPERIMENTS ABOVE THE CRITICAL TEMPERATURE

Experiment No.	Temperature, °C.	Pressure, atm.	Mixture (HCl: Propylene)	Time, hr.	Result
11	85	100	2:1	2	No reaction
12	80	105	2:1	2	No reaction
13	78	105	2:1	2	No reaction
14	75	100	2:1	2	No reaction

The above data show that within 5° C. of the critical temperature of the mixture no reaction occurs. Experiments at temperatures between 75° C. and the critical temperature give rather indeterminate results. The velocity of reaction in the liquid state follows a normal course. At 72° C., a 2:1 reaction mixture was found to give a reaction, but the curve shows acceleration to take place. Perhaps this is brought out most clearly by a graphic representation, Fig. 5, giving the amount of reaction for a liquid reaction, 50° C., and the reaction just above the critical temperature, 72° C. The interpretation of the forms of these curves is that just above the critical temperature the threshold condition exists in which the reaction is at all possible. The products of the reaction are much less volatile so that once the reaction starts, a condensing medium is formed and the reaction takes on a more normal course. Thus at 72° C. only the first part of the curve shows acceleration. A number of experiments between 75 and 70° C. gave this type of curve. The highest temperature at which a reaction was at all possible was 74° C.

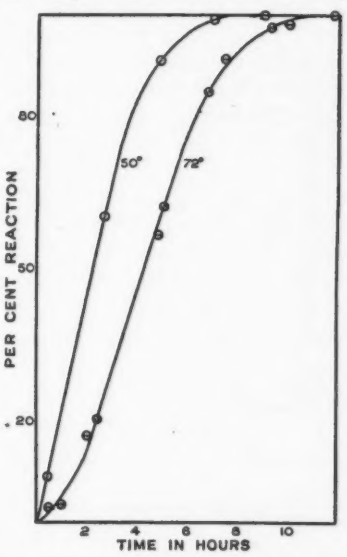

FIG. 5. *Typical reaction curves for reaction below critical temperature of both components and for reaction just above critical temperature of mixture.*

It is therefore shown by these experiments that no reaction takes place to within a few degrees of the critical temperature. Whether the change between

reaction and no reaction is less than a 4° C. interval remains to be investigated. It is certainly not greater.

The measurement of the velocity of reaction in the liquid state can be carried out very readily up to a temperature of 52° C. This was done and the results obtained are shown in Table V.

TABLE V

VELOCITY OF THE REACTION OF PROPYLENE AND HYDROGEN CHLORIDE IN THE LIQUID PHASE

Temperature, °C.	0	20	50
Velocity of reaction, % per min.	0.146	0.208	0.333

It is seen that the velocity of the reaction increases in a regular manner up to 50° C.

The interval between 50 and 70° C. presents an experimental difficulty which cannot be completely overcome with regard to the velocity of the reaction in the liquid phase. Due to the low critical temperature of hydrogen chloride, a gaseous phase is always present*. The procedure followed however in making an estimate was to measure the velocity of the reaction, irrespective of the fact that the gaseous phase was present, at 55 and 69° C. The velocity of the reaction increased from 55 to 69° to exactly the same extent as over a similar range just below 50° C. This would therefore indicate that the velocity of reaction in the liquid continues to increase right up to 69° C. In spite of the presence of the gas phase it is practically equal to that at 50° C.

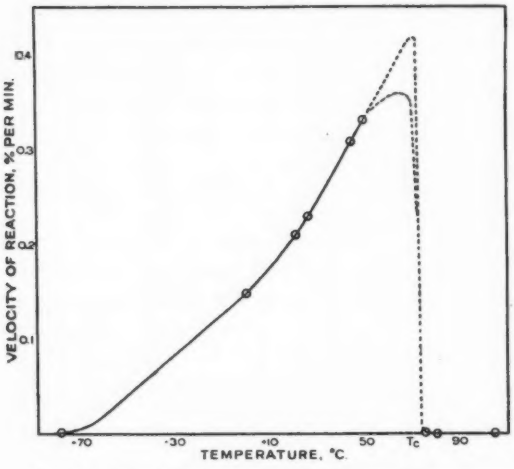

FIG. 6. *Reaction velocity over complete temperature range.*

It follows therefore that the velocity of the reaction in the liquid phase increases with rise in temperature until the near neighborhood of the critical temperature is reached. Perhaps the experimental results can be summed up most adequately by means of a velocity of reaction-temperature graph.

In Table VI are given the values for the amount of reaction per minute before the reaction has proceeded to half-value, at the various temperatures at which experiments have been carried out.

**The experiments were all carried out at pressures in the neighborhood of 100 atm. It may be possible that a homogeneous liquid phase could be obtained at much higher pressures, due to increased solubility of hydrogen chloride in the liquid phase.*

TABLE VI

VELOCITY OF THE REACTION BETWEEN PROPYLENE AND HYDROGEN CHLORIDE AT DIFFERENT TEMPERATURES

Temperature, °C.	−78.5	0	+20	+25	+45	+50	+75	+78	+80	85	+105
Velocity of reaction, % per min.	0	0.15	0.21	0.23	0.31	0.33	0	0	0	0	0

In the graph, Fig. 6, a curve is drawn through the experimental points and the dotted portions include an area within which the curve must lie. In the region between 50 and 74° C., measurements that would have the same quantitative significance could not be made. The two dotted portions indicate however the extremes within which the curve representing the reaction in a homogeneous phase must lie.

Discussion

It was pointed out in the introduction that a corollary to the hypothesis that a chemical reaction is dependent on either a molecular attraction between the reactants or where this does not exist, dependent on a catalyst, is that with rise in temperature the velocity coefficient of such a reaction might diminish and even become negative. A more detailed discussion of this is given by Maass and Sivertz (9, p. 2889). The question arises whether the results obtained in this research give evidence of such an inversion in the temperature coefficient It must be kept in mind that the hypothesis does not demand an inversion, but simply requires an influence contrary to the effect of activation, which latter may be so large as to mask the other influence. Therefore the absence of an inversion does not necessarily disprove the case.

Coming now to the experimental results; if the velocity coefficient of the reaction in the liquid state had shown a decided decrease at the higher temperatures, not much further need be said. All that has been shown definitely however is that in the liquid state the velocity of the reaction increases in the region where quantitative results are obtainable and becomes zero above the critical temperature, although no discontinuity in concentration takes place under the experimental conditions. It might therefore be concluded that the velocity coefficient of the reaction acts normally in the liquid state with change in temperature, and that the change in state precludes a reaction occurring. The hypothesis therefore which is justified is a modified form, and may be stated briefly as follows. *In the liquid, regional orientation takes place, especially where in a binary mixture the two species of molecules have a strong attraction for one another. This regional orientation is of great advantage in furthering the reaction. With rise in temperature the thermal agitation decreases the extent to which this regional orientation takes place. The increased activation of the reacting molecules with rise in temperature more than compensates for this, until the critical temperature is reached. At the critical temperature there is a very rapid decrease in regional orientation resulting in an equally rapid change in the velocity coefficient.*

It is realized that the above views may appear rather radical, as involving almost a discontinuity in state at the critical temperature. This might be considered contrary to the parallelism of the pressure-volume isothermals, one just above and one just below the critical temperature. The drastic change in regional orientation which is assumed to take place in the neighborhood of the critical temperature, while obviously of great influence in governing the reaction velocity, need not necessarily however greatly influence the pressure-volume relationships of the two isothermals mentioned above, and thus not give rise to an apparent discontinuity in state.

As an analogy to the above statement the phenomenon of the so-called "liquid crystals" may be mentioned. The turbidity and optical properties of these substances undergo a rapid change in a very small temperature region without any appreciable change in the other physical properties. This is due to a pronounced regional orientation (1, 2, 3) where exceptionally polar molecules are in question. The regional orientation in the liquid is a resultant orientation of the liquid molecules differing from the perfect and fixed orientation of the atoms in a crystal, inasmuch as the molecules retain their identity and rotational and kinetic energy in the case of the liquid. The average molecule in a certain group only is orientated with respect to its neighbors. The sharp change in turbidity in the case of the liquid crystals without corresponding sharp change in physical properties has been satisfactorily explained by Nernst (13) on the basis of a sudden change in regional orientation.

That regional orientation exists in all liquids to a greater or lesser extent is a possibility. That it exists in highly compressed gases is a further possibility. That a marked change in the extent of regional orientation is closely associated with the critical phenomena is the speculative viewpoint of the authors, inasmuch as it is useful in explaining the results of the experiments.

The ideas which have been developed are necessarily subject to modification in the light of further investigations. These ideas however, if nothing else, are useful in suggesting a considerable amount of research which is to be undertaken in this laboratory. Already a research is under way in which the critical phenomena are being investigated, and some interesting results have already been obtained with regard to the lag in equilibria in a one-component system at the critical temperature. The particular reaction between propylene and hydrogen chloride is to be examined in an inert liquid medium at temperatures above those reached in the present investigation, and also other systems are to be examined. The effect of the presence of other components on the course of the reaction is to be investigated. It may well be for instance that, where a number of simultaneous reactions occur, the fact that above a certain temperature some of them may cease, will be of considerable practical value.

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THE USE OF ARTIFICIAL ILLUMINATION FOR GRADING GRAIN[1]

By D. C. Rose[2]

Abstract

The first part of this paper is a description of artificial lighting units designed to give a suitable illumination for grading grain. Two types of illumination are being tried; first, the imitation of daylight by means of daylight lamps; second, the use of colored lights which emphasize the bad and good points in wheat. A combination of a mercury lamp, neon lamp and the General Electric type S1 sun lamp gives promise of being a satisfactory source of illumination of the second type. A grain grading unit of each type is being given a prolonged trial.

The second part describes experiments which were an attempt to find a more objective means of grading wheat. The light reflected from wheat of different kinds and different grades was analyzed both spectroscopically and by means of a photo-electric cell and light filters. In the spectroscopic measurements ultra-violet light was included. The results indicate a certain amount of selective reflection but the variations with the different grades are not of a nature which would be helpful in grading wheat.

There are two important reasons for the adoption of artificial illumination for grading grain. First, some difficulty has been experienced with the present practice in that a sample of wheat is sometimes given a different grade when examined at inspection points some distance apart. It is quite probable that many such disagreements in the grades are due to the differences in illumination. There is also a personal element which probably can never be entirely eliminated unless some physical means of grading are found. The use of a uniform type of illumination should reduce such errors to a minimum. Second, during rush seasons there is often delay at elevators and in transportation as the grain cannot be binned or shipped until it has been graded. Often in dull weather there are only a few hours of daylight suitable for examining the grain and costly delays occur. If artificial lighting were used these delays would be eliminated and in rush seasons grading could be carried on all night.

In this report are described lighting units which the author erected in the Laboratories of the National Research Council in Ottawa, with the object of having them tried out by grain inspectors. Two very different types of illumination show prospects of being satisfactory. A description is also given of an attempt to use other physical means of grading wheat.

Part I

Sources of Artificial Illumination

Requirements

The requirements for the illumination of a grain inspection table are slightly more exacting than, though not dissimilar from, artificial illumination problems where color matching is required. The requirements may be put under three

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Contribution from the National Research Laboratories, Ottawa.

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heads: (a) color or composition of the light, (b) intensity of illumination, (c) diffuseness of the illumination.

Of these three qualities it is difficult to say which is the most important. One usually assumes that for color comparisons the first is by far the most important, but there is considerable evidence that the other two are equally important and in fact there is some evidence that intensity is rather more important than exact color or composition of the light, provided it does not differ enormously from white light. This point will be brought out below.

The choice of a source of illumination was considered from two points of view. First, the imitation of daylight was obviously a step in the right direction since it is the type of illumination ordinarily used for grain inspection. Second, it was decided to try to produce an inspection cabinet or booth in which the illumination would come from lights of different colors, which would make the more important blemishes as well as the good points in wheat appear more prominently than with daylight.

The details as to the best intensity, diffuseness and color of light to be used for grading grain were obtained only after a number of lighting arrangements had been tried, so it seems advisable to describe at this point some of the lighting units tried and then discuss the requirements under the three heads given above. In order to make as little change as possible in the routine of grain inspecting, it was necessary to make the inspection table or space as nearly similar as possible to that already in use. The usual inspection table is a bench about two to three feet wide placed directly in front of a large north window. As far as the author is aware, there is no definite rule regarding the size or height of the window, but it must not be shielded by other buildings and must be sufficiently large to illuminate uniformly an area on the bench, two to three feet square, and with sufficient intensity for the inspector to be sure of his grade.

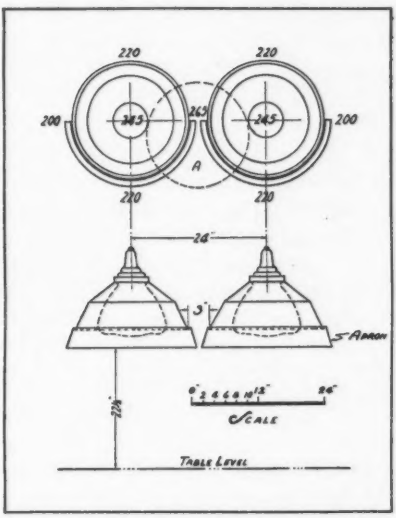

FIG. 1. *Daylight units consisting of two Ivanhoe Glassteel Diffusers with "Trutint" globes. The figures in the plan represent the approximate intensity on the table in foot-candles. The circle A is the best place to examine the wheat. The inspector should hold his head above the place marked A.*

Daylight Units

The most usual type of daylight unit is either an ordinary gas-filled incandescent lamp, the bulb being made of a special blue glass, or an ordinary clear glass bulb covered by a globe made of special blue glass. The object of

the blue glass is to absorb the excessive red and yellow rays produced by the incandescent filament. This, of course, reduces the efficiency of the lamp somewhat. There is an enormous number of such fixtures of various shapes and sizes on the market (1, p. 302), a large number of which would obviously be unsatisfactory. Before settling on definite units to be tried, a consultation was arranged with Mr. J. W. Bateman, Manager of the Lighting Service Department of the Canadian General Electric Co., Toronto. The experience gained by this Department was found to be invaluable and was largely responsible for the selection and arrangement of the lighting unit which will probably be most satisfactory for grain inspection.

For direct lighting units several types of metallic reflectors were tried, but the two most satisfactory were found to be, first, a pair of Ivanhoe Glassteel Diffuser fixtures (500 watts each) with a "Trutint" glass globe. Two such fixtures form one lighting unit arranged as in Fig. 1. Second, a pair of aluminium high mounting units (500 watts each) using Mazda daylight lamps gave a good diffuse illumination, but the color was not as good as with the Trutint globe, so it was not considered necessary to give them a prolonged trial. They are merely mentioned as being a second best. The Glassteel Diffusers were, however, set up in Winnipeg for a prolonged trial.

In order to obtain the required intensity, it was necessary to hang the Glassteel Diffuser fixtures about 22 in. from the table. With the fixtures at this height, a grain inspector, if he looked up, could see the bottom of the globe. This arrangement would cause a certain amount of glare in his eyes which it was desirable to eliminate; an additional apron was therefore added to the shade. This apron extended only half way around the fixture so that the inspector whose eyes are normally about on a level with the bottom of the apron would receive no glare from the opposite side of the shade.

Colored Light Sources

Examination of a sample of wheat under an intense mercury vapor lamp gave a surprising result. The green and frozen wheat showed up much more plainly than in daylight. Starchy kernels could also be distinguished though not quite as easily. The good grain was least prominent, which is not surprising as the mercury spectrum consists mostly of strong yellow, green, and blue violet lines. The frozen wheat seems to reflect a relatively higher portion of blue and violet light than good or starchy kernels. Hence this property is magnified by the excessive intensity of light of that color. Green wheat is made prominent by the intensity of the mercury green line, but this light alone would not do for grading as it suppresses the good points of the grain. The wheat was also examined by light from an intense neon lamp, which, being nothing but an intense red light, made all the wheat grains appear red, signifying a better grade than it appeared to be in daylight. This light alone would be useless for inspection purposes because the green wheat would not show up at all and frozen wheat could be distinguished only by its scaly surface.

Another source of illumination which has been receiving attention recently

is the General Electric Type S1 Sun Lamp. This consists of a mercury arc between tungsten electrodes. The electrodes become incandescent and the general illumination is not unlike a Mazda lamp, but includes in addition the mercury lines in the ultra-violet. The lamp is made in a special ultra-violet transmitting glass. The addition of some ultra-violet light to the illumination of an inspection table for wheat seemed a possible improvement due to the fluorescence it produces, though this detail was not investigated separately.

A large number of combinations of the following three lamps and daylight lamps were tried:

1. Cooper Hewitt Mercury Vapor Lamp M Tube Type, 110 volts a.c., 450 watts.
2. Cooper Hewitt Hot Cathode Neon Lamp, 110 volt a.c.
3. General Electric Type S1 Sun Lamp.

Finally an experimental grain inspection cabinet as shown in Fig. 2 was built.

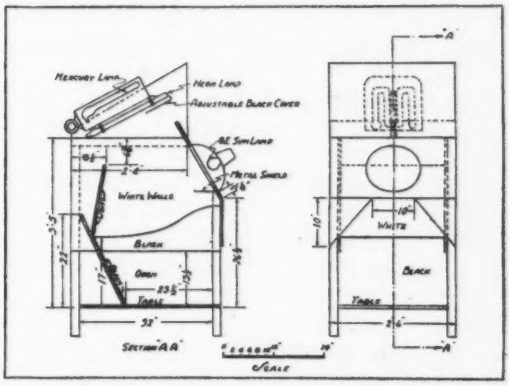

FIG. 2. *Inspection cabinet, containing mercury vapor lamp, neon lamp, and the General Electric Sun Lamp. Most of the supporting framework is omitted and only the important dimensions are given.*

The neon lamp was attached to the reflector containing the mercury vapor lamp. These were placed at the top of the cabinet, the light coming from them directly to the inspection table and being reflected from the white parts of the side walls. The General Electric Sun Lamp was shielded from the table and so placed that the lighting from it was indirect. The relative intensities of the three could be varied considerably by raising, lowering or tipping the reflector containing the mercury and neon lamps, covering the neon lamp, or varying the amount of white and black in the walls of the cabinet. The arrangement shown in Fig. 2 seemed most satisfactory and this unit was set up in Winnipeg for a prolonged trial.

Color and Intensity

As these two properties of illumination are to some extent complementary, especially with regard to this problem, it is difficult to discuss them separately.

First, with regard to color, it has been stated above that the lighting used normally for grain inspection is north skylight. The color temperatures of various types of daylight, as given by Luckiesh (2), are shown in Table I.

The light on the inspection tables in the inspection room of the Board of Grain Commissioners in Winnipeg was examined by the author for two days, and though no colorimetric measurements were made, some idea of the variation

TABLE I

COLOR TEMPERATURES OF VARIOUS TYPES OF DAYLIGHT

Type of daylight	Approximate color temperature, °K
Direct sunlight before 9 a.m. and after 3 p.m.	4400 to 5000
Direct sunlight 9 a.m. to 3 p.m.	5300
Sunlight plus light from hazy or smoky sky	5700
Sunlight plus light from clear sky	5800
Light from totally overcast sky	6400
Light from hazy or smoky sky	7000 to 8000
Light from clear blue sky	10000 to 20000

can be obtained from the above table. As the room is on the eleventh storey, and there are no neighboring buildings nearly as high, the light, with the exception of that from a ledge in front of the windows, comes from the north sky. On clear days with blue skies this has a color temperature of from 10,000° *K* to 20,000° *K*. On overcast days it might be about 6400° *K* as Table I indicates. The relative luminosity of violet and blue light at these color temperatures according to Luckiesh is 166 to 115 respectively; for green light, 116 to 105; for yellow light, 92 to 98; and for orange and red light, 75 to 93. The reversal in order of these figures gives an idea of the change in color even under best inspection conditions.

Further, it is quite possible that even a greater variation in color may be found. The intensity of illumination was measured at various times throughout the day for two days. The measurements were not very accurate as they were taken with a Macbeth Illuminometer with no color filter. However, the error in relative variation is certainly not greater than 6 or 8%. The results are plotted in Fig. 3. The increased intensity around 10 to 12 a.m. was probably due to a horizontal ledge, about 10 ft. wide, nearly level with the window sills and forming a part of the roof of the storey below. This ledge reflected a considerable quantity of direct sunlight which no doubt accounts for some of the increase in intensity at the top of the peak. Towards afternoon this ledge was in the shadow of the roof and the intensity dropped. The effect of this ledge would also decrease the color temperature of the light somewhat, thereby making it more yellow. Of course there is a natural variation in the intensity of daylight as well as in the effect of the sun on surrounding objects.

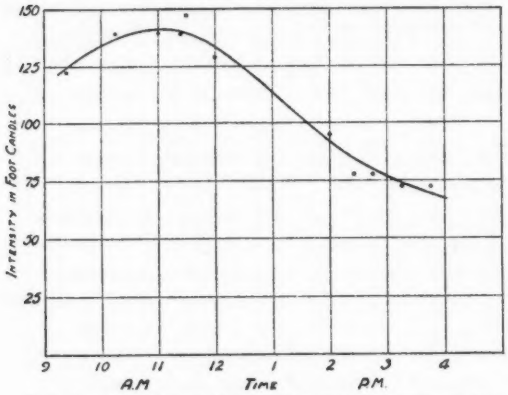

FIG. 3. *Intensity of illumination measurements on one of the grain grading tables in Winnipeg. Observations were taken on two clear days.*

Thomson (3) has made some measurements at the Manitoba Agricultural College in Winnipeg of the variations in intensity of daylight, that is, the total radiation coming from the sky in all directions. His results show curves not unlike that in Fig. 3. The intensity of north skylight shielded from the sun would not be expected to vary as much throughout the hours in Fig. 3 as total daylight, but the variations would be similar. He found that sometimes when the sky was patchy the intensity would increase for a short period as much as 50% above that for a clear day. Also the difference in intensity between a clear day and a dull day was very great. His curves were taken in winter and as they represent total daylight, cannot fairly be taken as representative of conditions on the grain inspection table. However, it may be concluded without doubt, that intensity variations occur in the illumination on the inspection tables which would probably be between a small fraction of the intensities shown in Fig. 3 to double the highest point on the curve. Such variations would exist under different weather conditions even between the hours of 8 a.m. and 4 p.m. during which most of the inspection work is carried out.

Thus we see enormous variations in both color and intensity of natural light, all of which would be avoided if artificial lighting were used entirely for grain grading.

It is well known that the color discrimination of the human eye depends greatly on the intensity of illumination on the object being observed. There is probably an optimum intensity at which the color discrimination as well as the form discrimination is a maximum. Form discrimination, that is, ability to see quickly the shape of the kernels as well as roughness of the surface, etc., is important in picking out green and frozen wheat. The intensity of illumination at which color and form discrimination are best for wheat inspection was found to be about 200 foot-candles. Anywhere from a little under 200 to 250 foot-candles seems equally good, though the best intensity probably varies somewhat with the individual. This intensity is considerably higher than is ordinarily used even for close work, but is compensated for by the low reflecting power of the wheat. Above 250 to 275 foot-candles the illumination is somewhat too bright for comfort. These observations of the best light intensity have been confirmed by engineers in the Lighting Service Department of the Canadian General Electric. It cannot be determined quantitatively with any great accuracy but the above figures were obtained by finding the intensity at which color and form discrimination was easiest for the observer. The best intensity would be expected to vary considerably with the object being examined and with the background, and probably is near the highest intensity at which there is no eyestrain.

The color of the lamps now being tried is not exactly that of north skylight. The light from the daylight units closely resembles the color of noon sunlight. The color of the light in the cabinets (Fig. 2) should not be compared with daylight as the illumination given by these lamps is mostly concentrated in certain wave-lengths. The Sun Lamp gives considerable illumination of the

continuous spectrum type but the light from the mercury and neon lamps is concentrated in the characteristic spectra emitted by these elements.

One of the problems in color-matching of any sort is to define exactly what light is to be used for a standard. Obviously, merely the term "daylight" will not do as it varies so much, and the term "white light" is also arbitrary and in itself is indefinite. The General Electric Company and others interested have done a great deal of work on this subject. They seem to have taken noon sunlight for the standard white light. Average noon sunlight has a color temperature from 5000° *K* to 5400° *K* and this color represents a good standard for white light. The color temperature of the Ivanhoe Trutint units similar to those being tried in Winnipeg is given by Luckiesh as 6200° *K*, so they are slightly more bluish than average noon sunlight. It is expected that this unit will prove satisfactory for grain inspection.

From data given above and the results of preliminary tests described below, it is evident that grain can be, and is being, inspected in a satisfactory manner in light of any color temperature from about 4500° *K* to 15000° *K*. It is also probable that the inspection cabinet described above, which gives mainly a line spectra source of light to which no definite color temperature can be applied, will prove satisfactory for grain inspection.

From these observations and those of others, it is becoming increasingly evident that in such work as the grading of wheat, the color of the light is if anything less important than its intensity and diffuseness, provided, of course, the color does not differ enormously from, say, noon sunlight. The author is convinced that light from an ordinary Mazda gas-filled lamp properly diffused and of the right intensity could be used for grain inspection, though it would be necessary for the inspector to undergo a period of training in order to grade correctly with such lighting. However, it is expected that, with the lighting units now installed in Winnipeg, no such period of training will be necessary, though it is not considered advisable to have an inspector constantly changing from artificial to natural light.

Diffusion

The diffuseness of the illumination has been mentioned several times without any definite measure of this quality being given. Actually no quantitative measure of diffuseness was made. It is advisable, where an assembly of small objects like grains of wheat are to be examined, to have the light coming in from all sides as well as from above. The main object of using two Glassteel diffusers instead of one was to give the correct diffuseness as well as a sufficiently large area of illumination (Fig. 1). The light coming to the inspection point marked comes mainly from above and from both sides. In the cabinet it was easy to have the light quite diffuse as the sources of illumination were large. The indirect components from the sides of the cabinet make the illumination so diffuse that no definite shadow can be seen when the hand is held as close as four or five inches from the table. The daylight units were almost as good.

Good diffusion is necessary for the examination of grain because the shape of

the kernels is very important, particularly in the detection of different kinds of wheat, foreign matter and immature wheat. A good diffuse light leaves no sharp shadow and no intense highlights where specular reflection takes place. The amount of specular reflection in wheat, although it has not been measured, is small. The condition of the surface of the kernel gives the indication as to whether or not it has been frost bitten.

Grading Tests with Artificial Light

While the author was in Winnipeg some tests were made using the two lighting units described in Fig. 1 and 2. The procedure was as follows:—

The grain was inspected and given a grade in the normal manner using natural daylight. This was considered to be the correct grade. Then the sample was passed on to the inspector using the inspection cabinet, without informing him of the grade already given. Here it was again graded and then passed on to a third inspector working under the daylight lamps. Forty samples of wheat were used, and a comparison of the sheets on which the grades were tabulated showed that the grades given under the daylight lamps were all correct. Thirty-four out of the forty were correct when graded in the cabinet. Two of the other six were doubtful, as they were on the border between 1 Northern and 2 Northern, but the inspector before finishing with them gave them the correct grade, leaving four still wrong. These were graded one grade too high. For this test the mercury and neon lamps were raised a little and tilted higher than in the position shown in Fig. 2, causing the light on the table to become redder and of about 150 foot-candles intensity. The lamps were returned to their normal position and the four samples re-examined and a correct grade was given. This, of course, can be considered as only a preliminary test. The units are being given a prolonged trial in Winnipeg and more complete reports will no doubt be available later.

Inspectors' Hours

The use of artificial light for inspection purposes is bound to cause a slightly greater nervous strain on the inspector, due more to the fact that he may be in a closed room or working at night than to any eyestrain caused by the lights. With this in view, a series of directions to inspectors have been drawn up by the author. It should be emphasized that these are only tentative and if grading is carried on at night, the periods of rest and hours of work adopted would have to be decided by those more familiar than the author with actual conditions in the elevators. These are merely offered as a working plan. The first two articles refer particularly to the cabinet containing colored lights. These should be adhered to strictly for the cabinet unit, if good results are to be obtained. However, an inspector should be able to change from daylight to the daylight lamps without much difficulty.

Directions to Grain Inspectors Using Artificial Light

1. Inspectors who grade grain by artificial light should use artificial light only and should not grade by any other light either during the day or night.

2. Should it be necessary to change an inspector from artificial light to daylight inspection or *vice versa*, the inspector should practice for a period to be

determined by the Board of Grain Commissioners or the chief inspector, with whichever light he is adopting before his word is taken as final.

3. An inspector should not grade continuously for a period greater than three hours without at least an hour's rest, and should not grade for more than two or at the most three such periods per day.

4. Recreation and fresh air are suggested between periods.

5. Under rush conditions these periods might be run up to four three-hour periods for a limited time, provided the inspector is in good health.

6. If it was found suitable, four two-hour periods with half-hour and hour intermissions taken alternately might be adopted. The best periods and hours can be found only by experiment and will probably vary for different men. Application of such a regulation would without any doubt improve the accuracy of daylight grading.

7. Inspectors should avoid looking directly at any of the lamps used for illumination, it being impossible to shield them completely.

8. If there is any tendency to eyestrain, the inspector should look around the room at distant objects for a few minutes. Five minutes' walk outside would be very good but if taken at night or on dark days, inspection should not be commenced for ten minutes after the intermission.

General Illumination of Grading Room

The general illumination of the inspection room is exceedingly important if artificial lighting is to be used successfully for grain grading. The requirements however are not very severe. The general illumination anywhere in the room should be between 20 and 40 foot-candles at the level of the table. This is rather a high level for artificial illumination but in order to reduce the intensity contrast between the inspection table and the rest of the room it should not be much less than 20 foot-candles. It must also be diffuse, for it is necessary to prevent bright highlights from any objects in the room. Indirect lighting with large illuminating surfaces would be best but is probably too inefficient for practical purposes. Glassteel diffuser units are probably next best, as the large globe and reflector diffuse the light sufficiently so that they can be looked at directly without straining the eyes. The whole room should be illuminated as uniformly as possible and the ceiling and walls down to about eye level should be white, and below that, some dull neutral color. The ventilation must be good and the room kept clean and attractive. This is sometimes very difficult in elevators, but if reliable grading is to be obtained considerable attention must be paid to comfortable working conditions for the inspector. This applies particularly to artificial lighting when used at night.

When grading is being done at night some eyestrain will be experienced when an inspector comes into the inspection room from outside. Owing to the high level of intensity necessary in the room, a man coming in from outside, having his eyes adapted to the dark, would find the light exceedingly brilliant and would be able to see nothing for several minutes. The strain involved in coming from darkness to a brilliantly lighted room is sometimes painful unless the individual is trained to shield his eyes and spend five or ten minutes in

getting used to the bright lights gradually. Inspection of wheat should not be attempted until the inspector has been in the lighted room for 15 to 20 min.

Location of Artificial Lighting System

The location of the lighting units, particularly the daylight units, is a question of some importance. It is not advisable to use them on the same table that is used for daylight inspection. If the hanging lights (daylight units) are near the wall, it will have considerable influence on the illumination of the table, as it acts as a source of indirect lighting. If hung in front of a window (*i.e.*, using the same table that is used for daylight inspection) a black blind drawn over the window at night would not interfere with the lighting but it would limit the inspectors' range of vision considerably. If when the inspector looks up from his work he can see only a wall immediately in front of him, fatigue will be noticed quicker than if the wall was ten or twelve feet away. It is for these reasons that it is recommended that the table for artificial light inspection be in the centre of the room rather than at the wall.

The position of the cabinet if it is adopted is not so important but should be arranged so that when the inspector turns from the cabinet to write down the grade his limit of vision should not be cramped by a nearby wall.

Part II

Grading by Physical Means

The object of these experiments was more to assist grain inspectors in grading wheat than to produce a purely physical method of determining the quality of wheat. Obviously the best method of giving a grade to a sample of wheat would be based on a milling and baking test, but such a test would require too much time. Actually the inspectors are trained to detect very quickly the imperfection which would spoil milling or baking and the process of actually giving a grade after the separation of foreign matter, etc., takes only about half a minute. It was hoped that an examination of the light reflected from the surface of a pile of wheat might yield a simple objective method of detecting the quantity of such blemishes as frozen grain, starch, etc. The results will be seen to indicate that there is insufficient selectivity in the reflection or scattering of light to be of any valuable help in estimating the amount of, say, frost bitten wheat present. The experiments indicate that the human eye is a great deal more sensitive in color and form discrimination than a photo-electric cell.

The problem was attacked by two methods. First, the intensity of the light reflected from various samples of wheat was measured by a photo-electric cell using Wratten filters to separate the colors. Second, the light reflected from the samples was examined spectroscopically and the spectrograms were measured photometrically to give the relative intensities at various wavelengths.

Measurements with Photo-electric Cells

The arrangement of the source of illumination and the box containing the

wheat and the photo-electric cell was usually as shown in Fig. 4. The source of illumination used varied with the color of light being studied. For the red part of the spectrum the General Electric Sun Lamp was used as it was found to operate very steadily. Owing to its construction and way it is connected with its regulating transformer, small variations in line voltage have but little effect on its intensity. For the blue and green light a mercury vapor lamp was used.

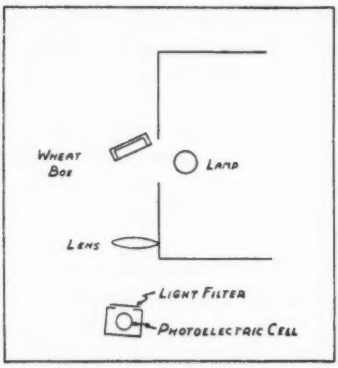

FIG. 4. *Arrangement of apparatus for measuring the intensity of reflected light from various kinds of wheat.*

The box containing the wheat was about 3 by 1½ by ½ in. and had a plain glass front which was kept clean. The box was so orientated that no specularly reflected light from the glass could reach the photo-electric cell. The light filters were placed directly in front of the photo-electric cell as shown.

The color sensitivity of the combination (photo-electric cell, filter and source of illumination) was checked by means of the Hilgar wave-length spectrometer, so as to make sure the light being measured was of the right color. The General Electric Sun Lamp, or for that matter any incandescent lamp, could not be used as a source of green and blue light as all the Wratten filters used transmit infra-red light and the photo-electric cell was sensitive some distance in the infra-red. At the time the experiment was performed no cells which were not sensitive in the red were available. Fig. 5 shows the results.

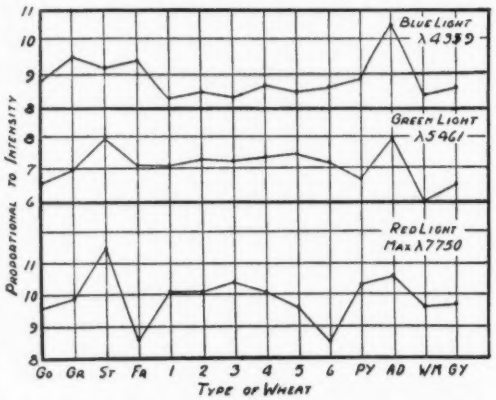

FIG. 5. *Variation in intensity of light of different colors reflected from various samples of wheat. Intensities measured by means of a photo-electric cell.*

The types of wheat sampled were: (a) Four samples picked by hand. They consisted of good wheat (Go.), practically perfect kernels; green wheat (Gr.), immature but not frost bitten; starchy wheat (St.), well filled starchy kernels; and frost bitten (Fr.), fairly well filled kernels with a heavy bran frost. (b) Then the six grades of wheat, numbered in the curves from 1 to 6, were tried. These samples were sent to the author by the inspection department of the Board of Grain Commissioners in Winnipeg. (c) Good samples of four other types of wheat were also used;

Parkers Yellow (PY); Amber Durum (AD); White Marquis (WM); and Garnet Yellow (GY).

An examination of the curves in Fig. 5 shows a variation of the type one would expect. First using red light, the good and green wheat reflect nearly the same quantity; the starchy, over 10% more and the frost bitten, less. With green light the relative intensity from starchy wheat was reduced and that from frost bitten wheat increased. With blue light (wave-length 4359Å) the intensity of light from starchy wheat was less than frost bitten. Owing to the yellow color of starchy wheat one would expect greater intensity of the reflected light in the red and green. An examination of the curves representing light from the six grades is also as would be expected. No. 1 Northern and No. 2 Northern were about the same, No. 3 Northern contained more starch and No. 4, 5 and 6 contained more frost bitten wheat as the grade became poorer. No. 6 was largely frost bitten wheat. The variation in intensity of green and blue light from the different samples is less than of red.

The other types of wheat show no variation of interest except that Amber Durum reflects relatively more light of all colors, particularly blue. It should be pointed out that the vertical axis of these curves represents the current in the photo-electric cell. The different curves cannot be compared as the different filters absorb different fractions of the incident light.

The accuracy of these curves is not very great. To check it a sample of wheat was put in the box and a reading taken, the wheat then taken out and poured in again and another reading taken; this was repeated many times and considerable variation in intensity was found. A set of readings obtained was as follows; 8.9, 9.6, 9.4, 8.5, 9.5. This shows a variation of over 10%, nearly as much as the variations in the curves in Fig. 5. However, as Fig. 5 represents the average of a large number of runs they are more accurate than that. This variation was not due to variations in the intensity of the illumination nor in the position of the box because these points were checked regularly.

Spectroscopic Measurements of Intensity of Reflected Light

Fig. 6 shows a plan of the apparatus used for this experiment. The object of using spectroscopic methods was to carry the investigation into the ultra-violet. However, some spectra were taken in the visible using a Hilgar wave-length spectroscope. The blackening of the plates was measured by a Zeiss microphotometer and the intensity of blackening plotted.

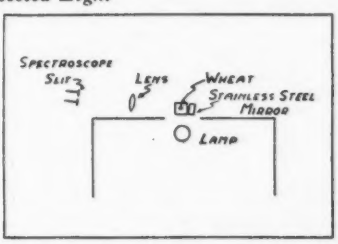

FIG. 6. *Arrangement of apparatus for photographing spectrum of reflected light from wheat.*

In Fig. 7, "A" represents the results in the visible part of the spectrum. Using visible light, the light from a small pile of wheat was reflected by a mirror and lens into the slit of the spectrograph. As high lights and low lights always exist in such a pile of wheat the lens was arranged so that the image of the

wheat did not focus on the slit. Different sources of illumination were used to bring out different parts of the spectrum. The results in Fig. 7 "A" were obtained with an iron arc and the General Electric Sun Lamp. The wavelengths bracketed were on the same plate. The ordinates are not given numerically as the plates were not calibrated. They represent the deflection of the electrometer fibre in the microphotometer. This bears a relation to the intensity of the light on the plates somewhat similar to a logarithmic curve, so the sensitivity of the method was considerably greater for faint spectral lines than for strong. The results agree with those taken with a photo-electric cell. The starchy wheat reflects relatively more red and yellow light than blue, while there is not so much difference between the other samples.

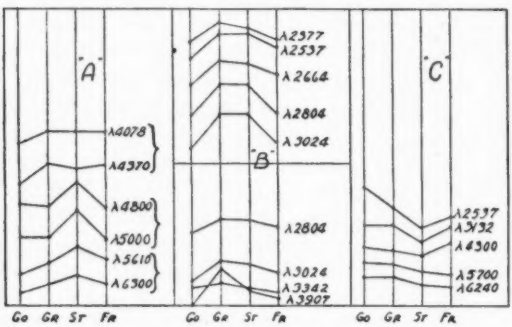

FIG. 7. *Variation in intensity of light of different colors, including the ultra-violet, reflected from samples of good, green, starchy and frost-bitten wheat. Intensities measured by spectral photometry. The wavelengths are given in Ångstrom units.*

Some variations show up with good and green wheat but not too much attention should be paid to these for reasons explained below, which make these spectroscopic results decidedly inferior to those taken with a photo-electric cell.

In Fig. 7, "B" and "C" represent the results taken with a quartz spectrograph. A quartz mercury vapor lamp was used for "B", and "C" was obtained partly with the same lamp and partly with a cored carbon arc. The results appear somewhat erratic. In this case a single grain of wheat of the type investigated was used as the sample. This was placed on a black cloth under the lamp and the light from it focused on the slit of the spectrograph by means of a stainless steel mirror and a quartz lens. The method used with visible light, that is, using a pile of wheat out of focus, did not work at all satisfactorily with the ultra-violet spectrograph and in fact the single kernel method was not much better. An examination of the spectrograph plate showed, first, that there was no definite band of selective reflection which would be useful in grading and, second, that even taking great precaution in getting uniform illumination of the wheat, owing to the rough uneven nature of the surface of the kernel, it was practically impossible to get a uniform portion of its image on the slit without using magnification which would make the exposures unreasonably long. As it was it took half an hour to obtain an exposure. The light was kept constant by directing a portion of it into a photo-electric cell and adjusting the current in the arc to keep the cell current constant.

Again in Fig. 7, "B" and "C", the zero of the ordinates is not shown and so only the general shape of the curves can be compared directly. For instance

the lines λ3907 and λ3342 show marked differences but actually the intensity variation was in about the same proportion, λ3907 being a much weaker line than λ3342. The difference in intensity of the light reflected from good and green wheat here would be about 15 or 20%.

As mentioned above, the slit was not uniformly illuminated, so the measured intensity of the lines would be different for different parts of the slit. An example of this is shown in the case of the lines λ3024 and λ2804 which were run through the microphotometer twice. The shape of the curve is in general the same but the variation in intensity is different. In Fig. 7, "C" further amplifies the result of irregular illumination of the different types of wheat. Here even with red light the intensity variation does not agree with that found when photo-electric cells were used. This is no doubt due to the fact that illumination of the starchy kernel was bad. To overcome this trouble and obtain consistent and accurate results would have involved the use of much more elaborate apparatus and as there is definitely no hope of using photometric methods of this sort for assistance in grading, further experimental work was not considered worth while.

The curves in "B" and "C" do, however, show some consistency with those in "A" and the measurements with a photo-electric cell. If the intensities of reflected light from the various types of wheat had been plotted in proportion to that reflected by, say, good wheat, the curves in Fig. 5 and 7 would be the same shape. Even in Fig. 7 "C" the relative intensity of light reflected from starchy and good wheat is greater for red light (λ6240) than for violet (λ4300). The line λ2537 appears in both "B" and "C" and the variation is quite different. This is also due to the difficulty of illumination.

Conclusion

Regarding the first part of this paper on the use of artificial light for grading, there is little to say except that, without doubt, artificial lighting can be used in a satisfactory manner for grading grain and from a purely physical point of view it should be better than natural light. One of the main objections to it, and probably the only real one, is psychological. Many individuals who are used to outdoor life and working under natural light find some discomfort in working in closed rooms using artificial light. While a grain inspector is not an outdoor man his training with natural light is so long and rigorous that a sudden change may cause real or imagined discomfort. This should be overcome to a large extent by keeping the room in which the inspector is working as pleasant as possible, well and uniformly illuminated and well ventilated and of course at comfortable temperatures.

Quite likely a considerable preference for artificial light would develop in inspectors who used it for some time. Many individuals who do a great deal of close work much prefer artificial light to daylight owing to its greater steadiness and the ease in locating the light where it is wanted.

The relative importance of intensity, color and diffuseness are fully discussed above so little more can be said here.

Regarding the second part, grading by physical means, the method tried did not give results which would be of any use. Consider the curve in Fig. 5 for red light. Supposing an unknown sample of wheat were put in the box and the current in the photo-electric cell gave a 10.1 for a reading. From the curve one would not be able to tell whether the sample were No. 1 Northern, No. 2 Northern, No. 4 or a mixturé of Amber Durum and White Marquis, or probably a dozen other kinds of wheat.

If the curve for grades from one to six had no reversal in slope then the results might have been useful and some sort of a colorimeter could be devised to help grading. The conclusions that can be drawn are that the grade is given more by the shape of the kernels and the nature of the surface than by the actual color. Of course color is extremely important but in the grading of grain it cannot be separated from form discrimination. In good, starchy and green wheat the color is more that of the inside of the kernel, while in frost bitten wheat, the color is more that on the surface.

Numerous blemishes other than those examined and numerous other types of wheat were omitted from the observations in order to correlate the results with known variables.

Other physical methods might be developed but it is doubtful if they would be any better. Grinding the grain before performing the experiments described in Part II might have produced more consistent results but they would not likely have been any more useful. If the exact properties of wheat which make it good for grinding and milling were better known, something more might be done to use physical means of grading. Chemical tests for protein, etc., take too long to be of any use and in any case the protein content of wheat does not seem to follow the grade in any regular manner. The only improvement that is obvious at present is the use of artificial light for examination.

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THE PROBLEM OF THE ELECTRICAL CONDUCTIVITY OF METALS[1]

By C. D. Niven[2]

Abstract

It is pointed out that mathematicians in their attempts to form theories of electrical conduction, do not lay emphasis on the fact that at low temperatures resistance, as a rule, does not vanish. In those cases in which it does, it vanishes suddenly. In view of this, the question arises as to whether the right model for conductivity in a metal is visualized. It is suggested that fundamentally a metallic atom is one in which the electron configuration is incomplete.

Ordinary conduction consists of a process whereby an electron jumps from one atom to another and remains with the atom it jumps to until it is in a sort of equilibrium with the motions of the electrons already on that atom. In the superconducting state, the electronic orbits of different atoms become synchronized so that when an electron leaves one atom, another electron automatically comes on to the atom to take its place. A study of the resistance-temperature curves as well as other points emphasizes the importance of structure in conductivity.

Introduction

The electrical conductivity of metals has of recent years been receiving much attention on account of the fact that existing theories do not explain experimental results. Sommerfeld, Houston and Bloch have published elaborate mathematical treatises on the problem. All of these writers assume in their treatment of the problem a free electron gas; Sommerfeld's treatise led to an expression for resistance which did not involve temperature at all, while the other two writers obtained formulas which agreed approximately with experiment, but implied that at the absolute zero of temperature the resistance would vanish. Hall has developed a dual theory in which some of the electrons are free to traverse long paths, several times the length of the mean free path, and some can pass only through the mean free path. Hall's theory evades certain difficulties but it must be admitted that no theory up to the present is entirely adequate.

There is no definite experimental evidence that at the absolute zero of temperature resistance vanishes; on the contrary, the resistance-temperature curves indicate that if resistance is to be zero at the absolute zero of temperature, the resistance will vanish suddenly as in the case of superconductors and not gradually disappear as the temperature is lowered. Again, there are certain marked peculiarities in different temperature-resistance curves which have to be accounted for. A study was made of the different temperature-resistance curves by plotting the results of experimenters and a classification was made. The curves fell roughly into three groups:— (i) Straight lines between 273 and 20° K, then curving very sharply; most divalent metals are in this group. (ii) Slightly curved all the way down; most monovalent elements lie in this group. (iii) Very curved; the common trivalent elements iron,

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aluminium, chromium and cobalt are in this group. This classification draws attention to the importance of atomic structure in the problem of conductivity and this is still further endorsed by the recent work on alloys at very low temperatures. By alloying bismuth and gold, a superconductor has been obtained. If bismuth gives up an electron to the gold atom in much the same way as a sodium atom gives up an electron to the chlorine atom in the formation of sodium chloride, the Bi-Au electronic configuration would become the Pb-Hg one; in the same way the Bi-Tl structure could become a Pb-Pb; or the Tl-Sb structure could become a Pb-Sn. It is of interest to note that the addition of bismuth to thallium raises the superconductivity threshold temperature by 3.9° K and thereby gives a temperature very close to that of lead.

The free electron theory does not make any provision for taking into account the structure of the atom. If it were successful in deducing a formula for the variation of resistance with temperature, that formula would have to involve quantities relating either to the crystal structure or the atomic structure of the metal, in order that the formula fit all the curves, for the curves of different metals are strikingly different. The rough classification of the curves decidedly indicates that atomic structure is the important factor. This indication is supported by the fact that a single crystal does not give a very different temperature-resistance curve from an aggregate of crystals of the same metal.

The fact is that far too simple a picture of the problem is usually visualized, very questionable assumptions are made and emphasis is not laid on the points on which our knowledge is very incomplete. Furthermore the subject of conductivity extends into almost every field in physics so that as soon as a theory is constructed with elaborate care to fit the facts disclosed in one field, the new theory is completely disproved by the facts from another field. It seems probable therefore that the best way of approaching the problem is to consider first the question—what is a metal? After forming clearly some sort of a picture of a metal, the next thing to emphasize is the vital difficulties in forming a theory. As our knowledge of experimental fact is very incomplete, any theory should be sufficiently elastic so that it may be supplemented as our knowledge grows.

What Is a Metal?

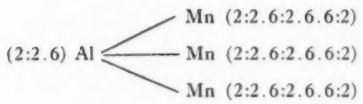
A glance at a list of the elements will show that the majority of them are metals. A metal is usually a base-forming element but some metals can also be acid forming—*e.g.*, chromium in chromates and iron in ferrocyanides. The metallic atom in these last cases acts as a central atom round which the other atoms are built; incidentally the metallic atom takes on one or more electrons when it acts as the central atom in an acid, and gives up one or more electrons when it acts as a base. An essential feature therefore for an element to be a metal seems to be that it can give up one or more electrons.

Hydrogen must be held as an exception to this statement of the rule for the reason that it forms molecules which act as atoms with closed shells and therefore molecular hydrogen cannot be looked upon as capable of giving up an electron when it is in the liquid or gaseous state. From this exception we

can see how to extend our ideas of a metal and postulate that a metal is a solid or liquid substance composed of atoms or groups of atoms with the electron configuration incomplete. By a complete configuration we mean one which has two electrons of azimuthal quantum number one and six of azimuthal quantum number two in the outermost configuration. Hydrogen and helium are special cases for obvious reasons.

In order to test this definition let us consider the elements which are non-metals. The rare gases and nitrogen must, by this definition, be non-metals. According to the latest system of grouping for homopolar molecules, oxygen and probably the halogens, in their diatomic state, can be considered as having completed electronic systems. This leaves sulphur, phosphorus and selenium to be accounted for; perhaps carbon, silicon, germanium and zirconium also should not be looked upon as entirely metallic. In the case of sulphur and phosphorus it is most probable that atoms combine to form molecules. Selenium and the elements in the carbon group lie on the border between metals and non-metals. At any rate it is clear that those elements with their systems most incomplete are the most metallic and also that elements like the rare gases which have completed systems are definitely not metals. If the incomplete system is a fundamental characteristic of a metal, one can easily understand why all attempts to explain intermetallic compounds on the laws of valency were useless, for if the compounds had obeyed the laws of valency they would have lost their metallic property.

As an illustration of the formation of alloys, the Heusler alloys might be considered. The structure of iron according to the usual notation for electronic grouping is described by:– 2 : 2.6 : 2.6.6 : 2. Now this is very strongly ferromagnetic, which is in all probability due to a peculiarity in the electronic structure; therefore, if the Heusler alloys can be regarded as having a like structure, the cause of their behavior might be considered as accounted for. If Mn_3Al is largely the cause of the ferromagnetism then the explanation is simple, for by withdrawing three valence electrons from the aluminium the manganese molecule can at once be given the structure of an iron molecule (A).



(A)

The magnetic alloy Mn_4Sn can be explained by the same reasoning; the tin would give up four electrons, one of which would be received by each of the four manganese atoms. Alloys like MnB, MnAs, MnSb, MnBi probably owe their ferromagnetism to a nickel-like structure. These examples are given merely to show how combination with another element may result in a compound being formed by an interchange of electrons—the structure is not completed as in an ordinary chemical compound like a salt but a new structure is formed as in the alloys just discussed. The difficulty in ascribing a structure to a metallic compound in general is that we have not the key as in the case of these ferromagnetic compounds.

Before leaving the discussion of metals it might be of interest to point out that certain oxides and sulphides of metals are conductors and therefore

might appear to form an exception at first sight, but this probably means that the resulting electronic configuration is not what would normally be expected—a sort of alloy is probably formed.

If we accept the picture of the metallic atom that has been described above, it is possible to proceed and discuss the conductor and the electric current. In the first place, a metal appears from crystal data to have atoms closely packed together (2). The question arises at once,—how do the various atoms influence each other by their proximity?—and here we have to admit our knowledge is quite inadequate. By photographing the absorption of a rare gas in the gaseous and liquid state McLennan and Turnbull (6) have shown that the electronic configuration of a gas does not radically change when the gas is liquefied, and if we can extend this result to atoms which have incomplete systems, we can consider the metal as made up of atoms of atomic structure similar to that shown by spectroscopy. Whether this is a permissible assumption or not is uncertain but probable. Here, therefore, is the first great difficulty that confronts one in developing a theory of conduction, and until more data are at hand to explain the forces which hold atoms together in the liquid and solid states one cannot hope to form a perfect theory. For the present, we must make the assumption that the atoms in a metal have the electronic configuration indicated by spectroscopy. This, as discussed above, is an incomplete system.

The Process of Conduction

In every theory of conduction one must assume that there are conductivity electrons in the metal. The motion of these electrons as a whole constitutes the current. Now, from what has been discussed above, a metallic atom appears to be one that has the power to take on an electron or give up one, and if, as Benedicks has suggested, the metallic atoms in a conductor act as transporters of electrons, one would expect a good conductor to be composed of atoms in which the outer shell was very incomplete and therefore suitable to transport a conductivity electron. Hence one should expect good conductivity in the alkali and noble metal group.

The close connection between electro-affinity and conduction was discovered quite a number of years ago by Benedicks (1). He found that the carrying capacity of an element, *i.e.*, the atomic conductivity divided by the characteristic frequency, was related to its position in the periodic table and suggested that the carrying capacity was concerned with electro-affinity. On the assumption which Benedicks made, of the atom acting as an electron carrier, the writer attempted, in a previous paper, to reconcile Drude's treatment of the electron gas applied to electrical conduction with Benedick's idea of an atomic carrier.

Although this may be done with a fair measure of success, there is this objection: it assumes that the atoms move. If the atoms in a metal are "close-packed" this movement must be exceedingly small. When the theory was subsequently followed up, a much more serious objection was discovered in connection with the transmission and reflection of light. If we consider the process of conduction to consist of a periodic motion of the charged atoms,

backwards and forwards, we must assume some periodic frequency; Benedicks used the frequency employed in Einstein's specific heat formula. In the treatment of the problem by a method analogous to Drude's treatment, the frequency turns out to be $\frac{v}{l}$, where l is the mean free path and v is the velocity of the atoms due to heat agitation. The ratio $\frac{v}{l}$ which has the dimensions of frequency, has to be put proportional to temperature if the resistance is to be put proportional to temperature. But if we take the formula for reflection, $100-R=\frac{200}{\sqrt{CT}}$, where R is percentage of light reflected, C is the conductivity and T is the period of the light, we find that the formula holds only down to a certain wave-length. This limiting value is presumably connected with the frequency of oscillation of the atoms. Therefore, if this limiting frequency for which the reflection formula holds does not change with temperature, the frequency of oscillation of the atoms during the process of conduction must be a constant and therefore Einstein's specific heat frequency must be chosen, and not the ratio $\frac{v}{l}$. If this reasoning is correct, the question arises as to what led to the mistake of choosing $\frac{v}{l}$. It seems clear that if the rate at which the atoms are to oscillate backwards and forwards is constant, then to explain the variation of resistance with temperature we must find some way of connecting either the number of transporting atoms or the number of electrons each can carry with the temperature, and the mistake entered in not taking into consideration that the number of carriers might change with temperature.

Obviously, to solve that part of the problem, some sort of statistical method is necessary, and that belongs to the field of the mathematical physicist. But there are very important points to decide before any attempt need be made to apply mathematics. In the first place, can a carrier carry more than one electron? How does the atom act as a carrier? In all probability only one electron at a time is carried by an atom and it is reasonable to suppose that the electron describes an orbit about the nucleus of the transporting atom. Now if there were thermal agitation present, then the orbit of the additional electron would be bombarded like the others and so a sort of equilibrium would be reached. Energy would thus be used up and this would account for the development of heat in conduction. In other words it is the motion of the outermost electrons of the transporting atom that is the important factor in determining the resistance; motion of these electrons is dependent on the thermal agitation, which in turn is dependent on thermal energy.

This conception is very much reinforced by a careful examination of Gruneisen's empirical formula, $\rho=\frac{T}{\theta}F\left(\frac{T}{\theta}\right)$, where $F\left(\frac{T}{\theta}\right)$ is the specific heat function of $\left(\frac{T}{\theta}\right)$ and θ is a characteristic temperature, sometimes a little different from the Debye characteristic temperature. $F\left(\frac{T}{\theta}\right)$ can be regarded as the rate of change of energy with a change in the modulus of distribution,

while the meaning of the factor $\frac{T}{\theta}$ is better understood by writing it $\frac{KT}{h\nu}$, in which case it is the ratio of two energies. Considering the term $\frac{T}{\theta}$, that is to say $\frac{KT}{h\nu}$, when KT is large compared to $h\nu$, then the resistance must be large. Now KT is associated with the energy of the atoms due to thermal agitation but the meaning of $h\nu$ is not known. The most that can be said is that it is the energy connected with a frequency ν. If there is anything at all in Benedicks' conception, it would appear as if $h\nu$ were the energy liberated when the electron jumps from one atom to the next. This quantity is fixed by the atomic and lattice structure of the metal and apparently if it is insignificant compared to the thermal agitation, it is difficult for the electron to get through or, in other words, the resistance is high.

But the resistance, according to Grüneisen, is also proportional to $F\left(\frac{T}{\theta}\right)$, *i.e.*, the rate of change of energy with change of temperature. Increase of temperature implies bombardment of the outer orbits of atoms by other atoms or molecules; the result of this bombardment is, of course, distortion of the electronic orbits if the atom cannot move and, in a "close-packed" solid, atomic movement must be small if present at all. The introduction of an additional electron to the system must also cause an increase in the distortion. Therefore in a sense the passage of an electron acts like thermal bombardment. Hence $F\left(\frac{T}{\theta}\right)$ may be looked upon as the increase in energy per unit of disturbance caused by passage of electrons.

The Effect of a Magnetic Field on Resistance

Exhaustive research along this line has been done by Kapitza. In his theoretical treatment of the subject (5) he shows that with large fields the resistance increases in proportion to the field; at low fields there is something that prevents the effect of the field appearing. The shape of the curve indicates that the resistance increase due to increase in field strength is of a similar nature to the resistance increase due to temperature. Kapitza has apparently proved that both the temperature-resistance curve and the magneto-resistance curve indicate a residual resistance at low temperature in the one case and at low field strength in the other. The full meaning of the term residual resistance is not yet quite understood.

Nevertheless in line with the speculations of other investigators, let us for the present consider the residual resistance as due to the construction of the metal, *i.e.*, how the metallic atoms are bound together, how the orbits are arranged and how their paths interlock. This conception is endorsed by the following facts: (a) that alloying always affects the residual resistance while it often may have very little effect on the slope of the temperature-resistance curve as a whole; (b) that tempering a piece of drawn or rolled metal will as a rule merely decrease the residual resistance.

When a conductor is placed in a magnetic field, it is reasonable to assume

that the orbits of the electrons, particularly in the incompleted shell, might be turned round and oriented in space differently from the way they are when there is no field. Since we know nothing about the orientations of orbits in a metallic mass, we need not attempt to discuss the effect of a field on them. We can say however that the action of the field on the orbits of the atom is to give them additional energy. This has been proved for single atoms by the theory of the Zeeman effect and, if resistance depends on the additional electron coming into some sort of equilibrium with the motion of the outer electrons of the transporting atom, it is not unreasonable to assume that it must come into equilibrium with the increased motion given to the outer electrons of the atom by virtue of the magnetic field. If such be the case, the increase in resistance should be proportional to the magnetic field and this is just what Kapitza found for strong fields.

When the field is small however this law is not true. Kapitza showed mathematically that an assumption is necessary which supposes that there is something about the atom that prevents the action of the field taking effect. With low fields this action predominates, while with strong fields it is negligible. If we extend the ideas which have been explained above, an explanation can be offered for this hypothesis of Kapitza's, for while the energy is undoubtedly given to the electrons, there is also a re-orientating of the orbits. These orbits themselves, before the field was put on, had a field which had an accelerating action on the electron, and the electron had to get into a state of equilibrium with this field: by being turned in their orientation they lost so much of that influence on the additional electron. The net effect therefore with a weak field was accordingly slight. Not until all the twist possible was given these orbits would the effect on the additional electron be proportional to the field strength. This suggests that in the atom itself there is electron motion, possibly resulting in a magnetic field with which the additional electron must come into equilibrium. This would suggest a residual resistance. When the field is put on, the orbits of the electrons are turned sufficiently to destroy this motion or "internal field", and leave merely the external magnetic field itself with which the additional electron must come into equilibrium. After the maximum amount of twist possible has taken place the resistance increase is proportional to the field strength.

Superconductivity

The residual resistance found by Kapitza's work and the residual resistance found by investigating the resistance-temperature curves at low temperature are, according to Kapitza, the same. The suggestion outlined above would require this. Now, since there is a small thermal motion even at 4° K as well as the electron motion, which for lack of a better name we have called the "internal field", it is obvious that the phenomenon of superconductivity cannot be accounted for unless it be no longer necessary for this equilibrium to be taken up by the additional electron. The logical conclusion is that there is no additional electron at all in the superconducting state. It would seem as

though instead of an additional electron jumping from atom to atom, in the superconducting state one of the electrons from one atom must jump to the next just at the moment when an electron from that atom has left. Such a process would imply a synchronizing of all the orbits. This synchronizing would of course be easily upset by the bombardment, due to thermal agitation, and one would also expect that a magnetic field would as a rule upset it. At the same time one would expect that it might be possible to orient the orbits in such a way that the synchronized state was more easily reached. This may account for the fact that adding bismuth to a superconductor (7) seems to raise the superconducting threshold temperature. One would rather expect that pressure would raise the superconducting threshold temperature. This experiment has not been performed and might present experimental difficulties.

The analogy of a set of gears might be used to explain the writer's idea in regard to the difference between the superconducting and the normal conducting state. In the superconducting state everything is timed like a set of gears in a mesh; in the normal state every atom acts as a gear, running separately or at least meshing only part of the time.

The explanation suggested above for the difference between superconductivity and normal conductivity gives a ready explanation of why at the superconducting temperature there is no discontinuity in the variation with temperature of specific heat, thermal conductivity, crystal structure or other physical property: for there is no difference in the structure and arrangement of the atoms—the electrons have merely got into an ideally synchronized arrangement.

Conclusion

The evidence shows that structure is of the highest importance in the problem of electrical conductivity. The term "free electron" implies that the bond between the so-called "free" electron and the nucleus is to be ignored. Any theory based on such an assumption can of course only be valid when the thermal agitation is so violent that the force exerted by this bond is insignificant compared with the force on the free electron when a neighboring electron strikes it. Grüneisen's formula as shown by Borelius (3) is in far better agreement with experiment than formulas given by the modern free electron theories, and it is hoped that this discussion will at any rate serve the purpose of emphasizing the importance of the Benedicks-Grüneisen viewpoint of conduction.

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FROST PRECIPITATION OF PROTEINS OF PLANT JUICE[1]

By R. Newton[2] and W. R. Brown[3]

Abstract

An exposure of 5 hr. at −7° C. caused maximum precipitation of the proteins of press-juice from leaves of unhardened winter wheat grown in the greenhouse. The value of sucrose added as a protection against precipitation increased with concentration up to about 8%. This concentration of total sugar is often attained by hardy varieties of winter wheat in the field. Sucrose and dextrose were about equally effective. By microscopic examination of smears caused by drops of juice, to which various quantities of dilute acid or base had been added, flowing across glass slides, it was ascertained that maximum precipitation occurred at about pH 5.1 and 7.3, respectively. Added sugar reduced the percentage precipitation by acid, base or "salting out." The addition of acid or common salt, insufficient to cause any immediate effect microscopically visible, increased subsequent frost precipitation; the addition of base or alkaline buffers decreased it. The removal of natural electrolytes by dialysis increased subsequent frost precipitation. Added sugar stabilized the juice proteins under all conditions. Precipitation by frost, acid or base are all irreversible. The coagula produced by different agencies acting on the juice have distinctive characteristics, which are illustrated. It is concluded that dehydration is the basic cause of frost precipitation, and that ice formation, acidity, salt concentration, and possibly pressure, are all contributing factors.

Introduction

Frost precipitation of the proteins of plant juice can easily be demonstrated, and has been thought to be the immediate cause of the frost-killing of plant tissue. While the exact mechanism of the precipitation has been in doubt, it has been clear that the withdrawal of water from the cells by ice formation in the intercellular spaces leads to an increased salt concentration and acidity of the sap. Either of these factors might bring about precipitation, and both have been suggested (2, 3, 12) as the agency.

On the other hand, there are factors which tend to stabilize the protoplasm, making it more resistant to disorganization by contact with concentrated sap. Two, chiefly, have been emphasized in the literature. The accumulation of sugars in the sap (4, 5, 12) and the splitting of the proteins to simpler, less readily precipitated forms (3, 12), during the hardening process, have been regarded as protective influences.

Protein-splitting has been investigated to some extent by the present authors (7, 8, 9) and will receive further attention in a later paper. Sugar accumulation has also been reported on, in the papers just cited, with the general conclusion that it tends to be greater during the winter in hardy varieties. This relation has been demonstrated by a number of workers, most convincingly by the long-continued investigations of Åkerman (1). Preliminary experiments already published (8) on the protection afforded by the sugars gave results in harmony with the views of other workers cited above.

The present paper reports a series of experiments done in 1924-25, on the frost precipitation of the proteins in the press-juice of winter wheat plants, as

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affected by salt concentration, acidity and sugar content, and by intensity and duration of exposure. By these experiments *in vitro*, it was hoped to gain a better understanding of the mechanism of precipitation and of the significance to be attached to the sugar concentrations naturally occurring in the field, as a protective adaptation of hardy plants.

Materials and Methods

Since it was desired to investigate the various factors affecting precipitation and the protective action of sugars, the press-juice of greenhouse plants was used (except in one or two specified cases) in order to obtain protein colloids in an unhardened condition and unprotected by any appreciable natural concentration of sugar. We have shown elsewhere (9) that proteins constitute the bulk of the colloidal material in the press-juice of wheat leaves, and that sugars in the juice of unhardened plants run less than 0.5% concentration. Two varieties of winter wheat were chiefly used: Minhardi, a hardy variety, and Fulcaster, a non-hardy variety. Two others were used in a few experiments: Buffum, a hardy variety, and Kanred, which is semi-hardy.

In sampling, the plants were cut off just above ground level. The material thus obtained consisted of leaves, since in no case had jointing of the plants taken place. The press-juice was prepared and handled with all possible precautions to prevent changes in the constituents other than those induced experimentally. The method of extracting the juice has already been described in detail (10).

Greenhouses controlled with regard to light and temperature were not available at the time of these experiments, and unavoidable fluctuations in these and other environmental conditions of the plants were no doubt responsible for much of the variability in the results. The plant material used in the various experiments is in most cases described in the tables with regard to the date of sampling, the age of the plants from date of seeding, and the content of total and coagulable nitrogen in the press-juice. The date affected the light and temperature conditions in the greenhouse, the temperature in winter running around 70° F. and in summer paralleling roughly the outdoor temperature, but at a higher level, especially on sunny days. The age of the plants affected the sensitivity of the proteins, and the concentration of nitrogen in the juice was found by Schaffnit (12) to affect the percentage of precipitation. The experiments in any one series were carried out simultaneously with portions of the same lot of juice.

Test tubes of 25 cc. capacity, with rubber stoppers, were used as containers for the juice during exposure. The freezing baths of ice slush and salt were well insulated and of large capacity. Where, however, the temperature of the bath changed appreciably during the period of exposure, the temperatures at the beginning and end are both stated in the tables. In the experiments on sugar protection, a tube of pure juice, another of juice with added sucrose, and in some cases a third tube containing juice with added dextrose were placed together in a freezing bath. When different concentrations of sugar were

being tested at a given temperature, the necessary number of tubes were exposed together in one bath. In the tables, the number of grams of sugar added to 25 cc. of juice has in each case been multiplied by 4 and recorded as a per cent.

After exposure, the samples of any one group were thawed in running water, and immediately whirled in a centrifuge for a standard time (10 min.). The supernatant fluid was decanted, and two samples of 10 cc. pipetted out for the determination of nitrogen. The values found were corrected where necessary to allow for changes in volume on the addition of sugar or other reagents. The percentage of nitrogen precipitated by the exposure was found by comparison of the total nitrogen in the supernatant fluid with the total and heat-coagulable nitrogen determined in the fresh press-juice.

The methods for determining nitrogen have already been described (9). The Kjeldahl method used for total nitrogen was not modified to include nitrates, and it was discovered that the added sugar reduced the nitrates and occasionally led to finding more nitrogen in the sugar-protected samples after freezing and centrifuging than in the original fresh juice. This difficulty had not been encountered with juice from field-grown plants, which contained sufficient natural sugar to reduce the nitrates. It was corrected in the latter part of the work with juice from greenhouse plants by equalizing all samples with respect to sugar content just before the Kjeldahl digestion.

The concentration of sugar usually employed in experiments involving sugar-protection, except when the effect of concentration was the point at issue, was either 8 or 4%. The former concentration is often reached naturally by the juice of field-grown plants in the late fall, and the latter concentration approaches the minimum found in hardy varieties at that time of the year (7, 8, 9). Both sucrose and dextrose were used in the first experiments, both being naturally present in considerable quantity in hardened wheat plants, and both being effective in reducing frost precipitation (8).

Effect of Temperature of Exposure on Frost Precipitation

The first experiments were designed to ascertain the effect of the temperature of exposure on the degree of precipitation, with and without sugar protection. Six series, lettered A to F, are recorded in Table I.

The experiments of Series A disclosed the surprising result that less precipitation occurred at the lower temperatures than at the higher ones. It was thought possible that at the lower temperatures complete congelation took place so rapidly that there was not time for dehydration of the colloidal particles to proceed to such a damaging extent as where the freezing took place more slowly. To offset this possibility, in Series B, C and D, the temperatures were lowered in steps, by transferring the tubes to progressively colder baths at intervals of an hour. For example, in the fifth experiment of Series B, the three tubes were first placed in a bath at $-4\frac{1}{2}°$ C. for an hour, then transferred to a bath at $-6°$ C. for the second hour, then to a bath at $-12°$ C. for the third hour, and finally to a bath at $-21°$ C. for $2\frac{1}{2}$ additional

hours, making a total exposure of 5½ hours. This method of exposure, however, did not alter the trend of the results to more than a slight degree, and an explanation must wait on further investigation.

The results of Series A to D show, with one minor exception, the greatest precipitation in the unprotected juice to take place at −7° C. This coincides with the first point of absolute killing of the tissue of both varieties used. The latter was determined by placing in test tubes fresh plants corresponding to those from which the juice was obtained, and immersing one tube in every freezing bath used, for the same length of exposure as the juice. At −3° C.

TABLE I

EFFECT OF TEMPERATURE OF EXPOSURE ON FROST PRECIPITATION, WITH AND WITHOUT SUGAR PROTECTION

Material	Exposure		Sample	Per cent total N pptd.	Per cent coag. N pptd.
	Time, hr.	Temp., ° C.			
A. Minhardi Feb. 27, 1924, plants 44 days old. N in 10 cc. juice: total, 53.7 mg. coag., 34.9 mg.	9	− 3	Juice only	36.9	56.7
	8	− 5 to −4½	Juice only	47.9	73.8
			Juice+8% S*	40.0	61.6
			Juice+8% D*	40.7	62.6
	8	− 8½ to −7	Juice only	50.6	77.9
			Juice+8% S	18.4	27.4
			Juice+8% D	19.0	29.2
	7	−11½	Juice only	37.4	57.6
			Juice+8% S	20.5	31.6
			Juice+8% D	24.2	37.2
	5	−21	Juice only	20.7	31.8
			Juice+8% S	6.9	10.6
			Juice+8% D	7.0	10.8
B. Fulcaster Feb. 29, 1924, plants 46 days old. N in 10 cc. juice: total, 40.5 mg. coag., 24.7 mg.	5	− 3	Juice only	28.6	46.9
			Juice+8% S	12.7	20.9
			Juice+8% D	12.3	20.2
	5	− 4½	Juice only	48.0	78.7
			Juice+8% S	33.0	54.2
			Juice+8% D	30.2	49.5
	1	− 4½	Juice only	56.0	70.6
	4	− 5 to −7	Juice+8% S	30.5	50.1
			Juice+8% D	31.3	51.4
	1	− 4½	Juice only	43.0	70.6
	1	− 6	Juice+8% S	19.0	31.2
	3½	−12 to −10	Juice+8% D	26.4	43.3
	1	− 4½	Juice only	36.1	59.3
	1	− 6	Juice+8% S	11.9	19.6
	1	−12	Juice+8% D	13.2	21.7
	2½	−21			
	5½	−21	Juice only	25.3	41.5
			Juice+8% S	6.9	11.3
			Juice+8% D	5.8	9.6

*S = sucrose; D = dextrose.

TABLE I—*Continued*

Material	Exposure		Sample	Per cent total N pptd.	Per cent coag. N pptd.
	Time, hr.	Temp., °C.			
C. Minhardi	5	0	Juice only	0.4	0.7
Mar. 5, 1924,			Juice+4% D	0.0	0.0
plants 51 days old.					
N in 10 cc. juice:	5	− 4½ to −3	Juice only	4.9	7.5
total, 47.4 mg.			Juice+4% S	0.0	0.0
coag., 30.8 mg.			Juice+4% D	0.0	0.0
	1	− 4½	Juice only	16.2	24.8
	4	− 8 to −7	Juice+4% S	0.0	0.0
			Juice+4% D	0.0	0.0
	1	− 4½	Juice only	4.9	7.5
	1	− 8	Juice+4% D	0.0	0.0
	3½	−12			
	1	− 4½	Juice only	1.6	2.5
	1	− 8	Juice+4% D	0.0	0.0
	1	−12			
	2½	−21			
D. Fulcaster	5	0	Juice only	1.7	2.6
Mar. 7, 1924,			Juice+4% S	1.6	2.4
plants 53 days old.			Juice+4% D	0.5	0.7
N in 10 cc. juice:					
total, 36.6 mg.	5	− 4½ to −4	Juice only	21.2	33.1
coag., 23.4 mg.			Juice+4% S	1.1	1.8
			Juice+4% D	2.2	3.4
	1	− 4½	Juice only	25.6	40.0
	4	− 7¼ to −7	Juice+4% S	4.5	7.1
			Juice+4% D	1.4	2.1
	1	− 4½	Juice only	11.3	17.6
	1	− 7½	Juice+4% D	0.3	0.5
	3½	−12			
	1	− 4½	Juice only	15.8	24.6
	1	− 7½	Juice+4% S	6.2	9.7
	1	−12	Juice+4% D	2.8	4.3
	2½	−21			
E. Fulcaster	5½	0	Juice only	13.6	20.9
May 8, 1924,					
plants 62 days old.	5	− 7	Juice only	13.5	20.7
N in 10 cc. juice:			Juice+8% S	0.5	0.7
total, 28.9 mg.					
coag., 18.8 mg.					
F. Minhardi	5	3	Juice only	16.7	29.7
May 13, 1924,			Juice+8% S	6.2	11.1
plants 69 days old.					
N in 10 cc. juice:	5	0	Juice only	26.1	46.6
total, 54.1 mg.			Juice+8% S	10.7	19.1
coag., 30.4 mg.					
	5	− 7	Juice only	30.6	54.4
			Juice+8% S	7.9	14.1

the plants of Minhardi were uninjured, but those of Fulcaster showed considerable damage; at $-4\frac{1}{2}°$ C. no injury to Minhardi was yet apparent, while Fulcaster was very seriously injured; at $-7°$ C. and all lower temperatures the plants of both varieties were killed outright. It is noteworthy that even under greenhouse conditions, which are not conducive to "hardening-off", the Minhardi plants retained some of their superiority in frost resistance. In this instance, it is reflected in the relative precipitation in the unprotected juice of the two varieties, but in other experiments this was not found to be a necessary relationship.

The experiments of Series C and D were carried out a week later than those of Series A and B, with plants from the same seeding, and apparently some change had taken place in the plant material in the interval, since the precipitations in C and D were all of a much smaller order of magnitude. Such a rapid change in the apparent sensitivity to frost of the cell proteins cannot be fully explained at present, though the sensitivity does appear to decrease with age, as will be shown later.

Series A and B were carried out with an added sugar concentration of 8%; Series C and D with 4%. At first glance it appears that the smaller concentration was actually a more effective protection than the higher concentration. In view of the altered reactions of the pure juice, however, such a conclusion is not justifiable. It is clear that the value of sugars cannot be stated in absolute terms of percentage protection, but only in relative terms, and that comparisons of the value of different concentrations of sugar or of different degrees or lengths of exposure must be based on experiments with a single lot of press-juice.

Sucrose and dextrose in the present experiments appeared to be of about equal protective value, variations of small magnitude occurring in both directions. To simplify subsequent experiments, therefore, only sucrose was used. For the same reason, the temperature of $-7°$ C. was adopted for all frost exposures, since it had been found most effective in precipitating the proteins.

The short Series E and F added at the end of Table I illustrate the unexpectedly high precipitation found occasionally in juice stored at 0° C. There would appear to be some effect of cold which operates injuriously even in the absence of ice formation. Potentiometric determinations of hydrogen ion concentration, made on other samples of juice at a later date, did not show any measurable change during 5 hr. storage at 0° C. A surprising feature in this instance is that as much precipitation occurred in the Fulcaster juice at 0° C. as at $-7°$ C., while in the Minhardi juice the difference in precipitation at these two temperatures was not large. The possibility of explaining the precipitation at 0° C. on the basis of enzyme activity seems to be precluded by the fact that less precipitation occurred in Minhardi juice stored at $+3°$ C. In passing it might be noted that this precipitation at 0° C. was always in greater evidence in the juice of older plants, and that added sugar always stabilized the juice considerably, whether frozen or not.

Effect of Length of Exposure on Frost Precipitation

Having found −7° C. an effective precipitating temperature, the next step was to determine the influence of length of exposure. It seemed possible that changes induced by frost might at first be reversible but in time become irreversible, and that this might have an important relation both to precipitation of the proteins *in vitro* and to frost-killing in the field. Four series of experiments on this point are reported in Table II. To simplify the records, the per cent coagulable protein precipitated has been omitted from this and later tables. While frost precipitation was probably restricted to nitrogen

TABLE II

EFFECT OF LENGTH OF EXPOSURE AT −7° C. ON PRECIPITATION OF UNPROTECTED AND SUGAR-PROTECTED JUICE

Material	Hours exposure	Per cent nitrogen pptd.	
		Juice only	Juice+8% sucrose
A. Fulcaster	1	4.4	4.1
May 8, 1924,	2	8.0	4.3
plants 62 days old.	3	9.1	1.7
N in 10 cc. juice:	4	8.2	0.0
total, 28.9 mg.	5	13.5	0.5
coag., 18.8 mg.	6	12.6	1.7
B. Minhardi	1	7.6	0.8
May 13, 1924,	2	10.3	1.1
plants 69 days old.	3	17.5	1.6
N in 10 cc. juice:	4	26.9	4.0
total, 54.1 mg.	5	30.6	7.9
coag., 30.4 mg.	6	37.8	24.4
C. Minhardi	1	8.2	0.1
June 15, 1925,	2	25.5	0.5
plants 16 days old.	3	40.4	1.8
N in 10 cc. juice:	4	44.7	2.5
total, 58.2 mg.	5	51.3	1.1
coag., 39.2 mg.	6	55.6	2.3
	7	—	5.0
D. Fulcaster	1	26.6	3.1
July 3, 1925,	2	35.0	4.1
plants 16 days old.	3	50.3	7.2
N in 10 cc. juice:	4	48.7	1.5
total, 37.0 mg.	5	51.7	5.4
coag., 20.1 mg.	6	58.8	3.8
	7	55.8	6.1
E. Minhardi, March 19, 1925, mixed plants 30 and 50 days old. N in 10 cc. juice: total, 64.3 mg. coag., 44.4 mg.	24	16.5	9.3
F. Fulcaster March 19, 1925, mixed plants 30 and 50 days old. N in 10 cc. juice: total, 49.2 mg. coag., 32.5 mg.	24	47.4	35.0

in the colloidal state, and the coagulable protein should therefore have more significance than the total nitrogen, the per cent precipitation of the former may readily be calculated, if desired, from the data now included in the tables.

Series A and B in Table II should be moderately comparable, since the plants used were of about the same age and grown under the same conditions. The juice of Fulcaster, the non-hardy variety, was considerably more dilute than that of Minhardi, a common distinction between these varieties, no doubt connected with their relative frost hardiness which tends to express itself under all conditions. In this instance, the Fulcaster juice appeared very stable, and might be thought to support Schaffnit's finding that precipitation is directly related to concentration of protein (12). But our own experiments do not permit any such generalization. It is contradicted in fact by the results of Series C−D and E−F in the same table. It has already been suggested that at least until such time as the experimental plants can be produced under standard conditions, controlled in all important respects, each lot of press-juice must be regarded as largely a law unto itself.

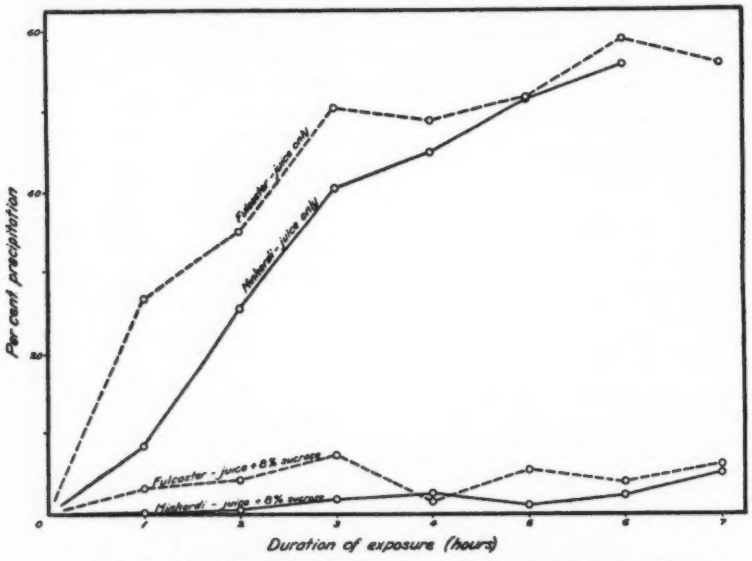

FIG. 1. *Precipitation of nitrogen in unprotected and sugar-protected juice at −7° C., in relation to length of exposure.*

For Series A, B and C, control plants in test tubes were immersed in the freezing baths with the test tubes of juice. The leaves of Fulcaster in Series A survived 2 hr. at −7°C., after which they were killed. Leaves of Minhardi in Series B showed only slight injury after 5 hr. exposure; in Series C they were killed in 2 hr. Comparing Series A and B, there is obviously no correspondence between the sensitivity of the leaves to frost-killing and of the juice to frost precipitation. But such comparison is scarcely justifiable, for the reason

given in the last paragraph. While great caution is necessary in comparing any two lots of press-juice, it is seen in Series B and C, in which only one variety comes in question, that frost precipitation is greater in the juice from the more sensitive leaves. When examination is confined to the results of one collection of one variety, either in Table I or Table II, there is found to be a direct relation between injury to the leaves and precipitation of the proteins, provided (Table I) the temperature does not go below −7° C.

There is some indication in Table II that the protective value of sugar may fall off with time. This is most pronounced in Series B, and is suggested again by the single cases of long exposure listed in Series E and F. Series C and D were done at a later date, after it had been found by special experiments that the younger plants were better suited to our purpose, and the results are rather more regular and consistent. They are presented graphically in Fig. 1, in which it is seen that precipitation increased rapidly with length of exposure up to 3 hr., and after that more slowly. The protective effect of the sugar is also clearly seen.

The early experiments were made the basis of using 5 or 6 hr. as a suitable length of exposure in most subsequent experiments.

Effect of Sugar Concentration on Frost Precipitation

The sugar concentrations used in the first experiments were based on those found in the juice of winter-hardened plants in the field. It seemed desirable to examine more particularly the significance of concentration by comparisons over a wider range. Experiments to this end are reported in Table III.

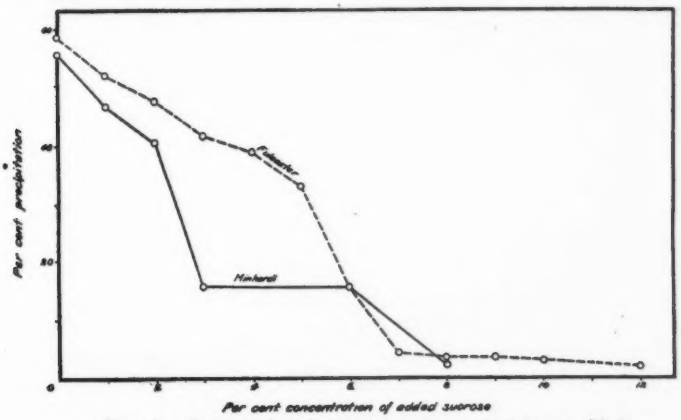

FIG. 2. *Precipitation of press-juice nitrogen in 6 hr. at −7° C., in relation to added concentration of sucrose.*

Portions of the same lots of press-juice used for the experiments reported in Table II were used for those in Table III. Attention has already been directed to the characteristic difference in nitrogen concentration of the juices of the two wheat varieties, and to the association of greater stability with a

more dilute condition in Series A-B but not in Series C-D. Regardless of these differences in other properties, all juices reacted in much the same way to the addition of sugar in various concentrations. Protection increased with concentration up to 8%. This is shown again in Fig. 2, plotted from the results of Series C-D.

TABLE III

EFFECT OF ADDED SUGAR CONCENTRATION ON PRECIPITATION AT −7° C.

Material	Hours exposure	Per cent sucrose added	Per cent nitrogen pptd.
A. Fulcaster	5	0	13.5
May 8, 1924,	5	2	7.9
plants 62 days old.	5	4	2.3
N in 10 cc. juice:	5	6	1.1
total, 28.9 mg.	5	8	0.5
coag., 18.8 mg.	5	10	0.6
B. Minhardi	5	0	30.6
May 13, 1924,	5	2	30.0
plants 69 days old.	5	4	17.8
N in 10 cc. juice:	5	6	17.4
total, 54.1 mg.	5	8	7.9
coag., 30.4 gm.	5	10	8.9
C. Minhardi	6	0	55.6
June 15, 1925,	6	1	46.5
plants 16 days old.	6	2	40.5
N in 10 cc. juice:	6	3	15.7
total, 58.2 mg.	6	6	15.7
coag., 39.2 mg.	6	8	2.3
D. Fulcaster	6	0	58.8
July 3, 1925,	6	1	51.9
plants 16 days old.	6	2	47.4
N in 10 cc. juice:	6	3	41.6
total, 37.0 mg.	6	4	39.0
coag., 20.1 mg.	6	5	33.0
	6	6	15.5
	6	7	4.6
	6	8	3.8
	6	9	3.8
	6	10	3.1
	6	12	2.0

Just why the increase in sugar protection should cease rather abruptly at 8% is not clear, unless there be a small proportion of the protein of a nature not amenable to stabilizing by sugar. This seems unlikely, since in some other experiments (Table I) apparently complete protection was obtained. It might be thought that higher concentrations would perhaps exercise a dehydrating influence on the proteins, especially since the effective concentration would be very much increased by the crystallizing of water on freezing the juice. This suggestion again seems untenable, since we have found (Table IX) that sugar reduces "salting out", when its effect should be additive rather than inhibitive if it acted as a dehydrating agent. It is significant, in any case, to find that the concentration required to give maximum protection against protein precipitation is not greater than that often attained by hardy varieties of winter wheat in the field.

Effect of Age and Condition of Plants

Because of the variability in press-juice properties encountered in the early experiments, some of the factors which might be responsible were investigated, particularly the effect of age of plants. The juice of field-grown plants usually increases in concentration of both total solids and nitrogen with age (9) while that of greenhouse plants, within the limits of age observed in these experiments, does not do so. The greenhouse juice is generally more dilute than the field juice, at any stage, but fluctuates up and down with temperature and moisture conditions. It might be supposed that if conditions were held constant the properties of the juice would also remain constant. There is some evidence, however, that the sensitivity of the proteins to frost decreases with the age of the plants.

TABLE IV

EFFECT OF AGE OF PLANTS ON PRECIPITATION OF UNPROTECTED AND SUGAR-PROTECTED JUICE

Variety and exposure	Date	Age in days	Mg. N in 10 cc. juice		Per cent nitrogen pptd.	
			Total	Coag.	Juice only	Juice + 8% sucrose
Minhardi	Mar. 2	13	47.1	—	35.6	—
Exposed 5 hr.	Feb. 13	16	44.1	29.1	56.3	0.0
at −7° C.	Mar. 9	20	54.3	—	48.7	0.2
	Feb. 20	23	44.9	23.3	42.2	0.0
	May 13	69	54.1	30.4	30.6	7.9
Fulcaster	Mar. 4	15	40.9	25.4	48.3	—
Exposed 5 hr.	Feb. 13	16	38.5	25.0	54.1	0.0
at −7° C.	Feb. 20	23	40.3	22.5	52.8	0.0
	May 8	62	28.9	18.8	13.5	0.5
Minhardi	Feb. 13	16	44.1	29.1	0.0	0.0
Exposed 5 hr.	Feb. 20	23	44.9	23.3	3.1	0.0
at 0° C.	May 13	69	54.1	30.4	26.1	10.7
Fulcaster	Feb. 13	16	38.5	25.0	0.3	0.0
Exposed 5 hr.	Feb. 20	23	40.3	22.5	5.7	0.0
at 0° C.	May 8	62	28.9	18.8	13.6	—

In Table IV are gathered the results of a number of experiments on precipitation at −7° C. and at 0° C., the data for each temperature and variety being arranged in order of age of plants. The collections of February 13 and 20 were made from one seeding, those of March 2, 4 and 9 from another seeding, and those of May 8 and 13 from still another. The diversity of growth conditions in the greenhouse at these various times is reflected to some extent in the fluctuating nitrogen concentration in the juice. This however shows no consistent relation to the degree of precipitation, which seems rather to be related to the age of the plants. The juice proteins show a tendency to become less sensitive to frost but, on the other hand, less stable at 0° C., as the plants advance in age.

To reduce the variability of the results, the practice was adopted of using as far as possible only young plants, in a state of full turgor at the time of cutting. The turgidity affects of course the concentration of the juice.

Effect of Adding Acid, Base, and Salt on Precipitation without Freezing

It seemed that the possible role of acidity and salt concentration in frost precipitation could in certain aspects be investigated best by experiments on the effect of these factors on unfrozen juice, and on the relation of sugar to their activity. Harvey (3) titrated with $N/10$ H_2SO_4 and $N/10$ NaOH, cabbage juice obtained from the midribs and from the rest of the leaves, having an original pH of about 5.8 and 5.6 respectively. He reported instantaneous precipitation with acid at about pH 5.1 in the midrib juice and pH 4.3 in the other juice, and with alkali at pH 6.6 in both juices. All of these points lie on the acid side of neutrality. The range between the precipitation points he concluded was the optimum hydrogen ion concentration for holding the proteins in solution in the sap. Since the increased hydrogen ion concentration required to precipitate the proteins by titration was closely paralleled by that which he found in the fluid portion of juice precipitated by freezing, when the ice crystals were removed, he concluded further that the increased acidity was the active agent in both cases, and that the increased salt concentration on freezing was insufficient of itself to cause precipitation.

Titrating wheat plant juice with dilute sulphuric acid and sodium hydroxide, we found the precipitation took place gradually over a range of about one pH unit, and to determine the maximum point developed the following technique. To a definite volume of juice in a test tube kept in an ice bath at 0° C., the reagent was added in definite increments. With 20 cc. of juice the increment of reagent was usually 0.5 cc. After each addition the juice was thoroughly stirred with a glass rod, and one drop allowed to run across a glass microscope slide fixed at an angle of 45°. A microscopic examination of the series of smears thus obtained made it possible to determine the point of maximum coagulation. The corresponding hydrogen ion concentration was determined upon another portion of juice to which was added a volume of reagent equal to that which brought the first portion to the point of maximum precipitation. All determinations of hydrogen ion concentration were made with a Leeds and Northrup type K potentiometer and a bubbling hydrogen electrode.

TABLE V

MAXIMUM PRECIPITATION POINTS AT 0° C. FOUND BY TITRATION WITH ACID AND BASE

Variety	Date 1925	Age in days	Mg. N in 10 cc. juice		Reagent added to 20 cc. juice		pH of juice	
			Total	Coag.	Kind	Cc. required	Original	At max. pptn.
Minhardi	Mar. 2	13	47.1	—	$N/5$ H_2SO_4	2.5	5.95	5.02
					$N/5$ NaOH	2.5	5.95	7.28
Fulcaster	Mar. 4	15	40.9	25.4	$N/5$ H_2SO_4	2.0	5.98	5.10
					$N/5$ CH_3COOH	3.0	5.98	5.02
Fulcaster	Mar. 13	24	—	—	$N/5$ H_2SO_4	2.5	6.00	5.16
					$N/5$ CH_3COOH	3.5	6.00	5.03

The results of some experiments of this kind are given in Table V. They indicate the precipitation points to lie at about pH 5.1 and pH 7.3. These can be considered only as approximate, since the acid and base were added in 0.5 cc. increments. More precise titrations may show the points of maximum precipitation with acid, which now appear similar for both varieties of wheat, to be identical. The precipitation occurs in stages corresponding to the hydrogen ion concentration. A series of smears representing an increasing number of additions of acid shows first a granulation which grows by steps to a distinct coagulum. After the maximum precipitation point is passed, the coagulated particles begin to disperse, and chemical action begins to show itself in the form of discoloration of the juice. Five stages in this process are shown in Fig. 4, *a-e*, made by tracing circular portions, 1 mm. in diameter, projected on a glass screen by a micro-projector.

When dilute sodium hydroxide is added to the juice, the precipitate is usually indistinct and always much less general than that caused by acid. In one experiment, dilute ammonium hydroxide was tried, but caused no precipitation. An interesting point seen in the experiment with Minhardi juice (Table V) is that it took the same volume of *N*/5 acid or base to bring about precipitation. Since the hydrogen ion concentration of the juice was not halfway between the two precipitation points, but nearer to the acid one, it would appear that it was more strongly buffered against acid than against base.

It is also of interest to note that of the two collections of Fulcaster, the one with older plants yielded a more strongly buffered juice, requiring more sulphuric or acetic acid to bring about the same change in pH, than the juice of the younger plants. This may have a bearing on the greater sensitivity to frost of the juice proteins of the younger plants noted in the preceding section.

The results of the titration experiments do not support Harvey's conclusion that the whole zone between the precipitation points represents optimum conditions for solution of the proteins, with instantaneous precipitation marking the limits. A relatively slight change in the hydrogen ion concentration of wheat juice, caused by adding acid or alkali, is sufficient to induce granulation which, however small, indicates incipient precipitation.

TABLE VI

HYDROGEN ION CONCENTRATION OF PRESS-JUICE OF FIELD-GROWN PLANTS AT DIFFERENT AGES

Variety	Date of collection	Age in days	pH
Kanred Seeded June 9, 1925	June 22	13	5.60
	June 29	20	5.34
	July 6	27	5.31
	July 13	34	5.38
	July 20	41	5.32
	July 27	48	5.00
	Aug. 3	55	4.87

The precipitation points found in wheat juice do not coincide with those found by Harvey in cabbage juice, except in the case of the cabbage midrib juice, which precipitated at about pH 5.1. This is not surprising, since we have found that even wheat plants must vary in their precipitation points when grown under different conditions. In Table VI are given the results of a series of determinations of hydrogen ion concentration of field-grown plants of Kanred wheat at progressive ages. If the properties of the press-juice of these plants had been the same as those of the greenhouse plants used in the experiments of Table V, it is clear that the juice of the last two collections should have precipitated spontaneously, whereas nothing of the sort occurred. Whether the isoelectric points of the proteins were actually different, or whether their stability was attributable to other factors, cannot now be stated.

Using the above method of finding the point of maximum precipitation, a number of quantitative experiments were made on the amount of precipitation caused by titration of the juice held at 0° C. The juice was centrifuged after titration, the nitrogen determined in the supernatant fluid, and the per cent precipitation found by difference. The results are presented in Table VII.

TABLE VII

MAXIMUM PRECIPITATION OF UNPROTECTED AND SUGAR-PROTECTED JUICE AT 0° C. FOUND BY TITRATION WITH $N/5$ H_2SO_4 AND $N/5$ NaOH

Variety	Date 1925	Age in days	Mg. N in 10 cc. juice		Reagent	Per cent nitrogen pptd.	
			Total	Coag.		Juice only	Juice+8% sucrose
Minhardi	Feb. 13	16	44.1	29.1	Acid	63.3	48.3
					Base	21.6	—
Minhardi	Feb. 20	23	44.9	25.3	Acid	61.5	53.7
					Base	7.8	0.0
Fulcaster	Feb. 20	23	40.3	22.5	Acid	64.9	47.9

The per cent nitrogen precipitated by acid was closely similar in the three cases, amounting to nearly two-thirds of the whole. A calculation will show that this represented about 109% of the coagulable protein in the second case and 116% in the third case. Evidently some of the non-coagulable proteins were carried down in the acid precipitate. The precipitation by the base was very much less, suggesting that the bulk of the proteins had their isoelectric point on the acid side. The addition of sugar afforded significant protection against the action of both acid and base. This apparently was not because of any effect on the reaction of the mixture, since a few determinations of the pH showed in all cases that of the pure juice and of the juice plus sucrose to be identical, even after the samples had stood for two days.

A modified form of experiment was carried out, in which the juice was not titrated but had added to it definite quantities of dilute acid or base, after

which it was allowed to stand 6 hr. at 0° C. It will be remembered that in earlier experiments the proteins had shown a considerable tendency to precipitate at this temperature (Table IV). The results given in Table VIII, and

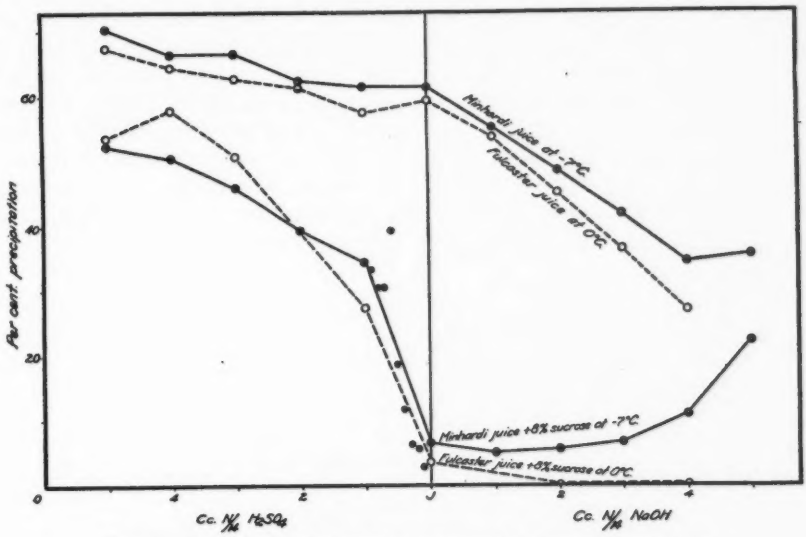

FIG. 3. *Precipitation of nitrogen in unprotected and sugar-protected juice in 6 hr. at 0° C. and 5 hr. at −7° C., as affected by various quantities of acid and base added to 25 cc. juice.*

shown graphically as part of Fig. 3, indicate that the hydrogen ion concentration has a profound effect on this reaction. Evidently this factor should be taken account of in any experiments on the precipitation of juice, since we have seen (Table VI) that it may vary considerably in different collections.

TABLE VIII

EFFECT OF ADDING ACID AND BASE ON PRECIPITATION OF UNPROTECTED AND SUGAR-PROTECTED JUICE IN 6 HR. AT 0° C.

Material	Reagent added to 25 cc. juice		Per cent nitrogen pptd.	
	Kind	Vol. in cc.	Juice only	Juice+8% sucrose
Fulcaster June 17, 1925, plants 16 days old. N in 10 cc. juice: total, 51.3 mg. coag., 34.9 mg.	*N*/14 H_2SO_4	5	67.3	53.4
	N/14 H_2SO_4	4	64.3	57.8
	N/14 H_2SO_4	3	62.7	50.8
	N/14 H_2SO_4	2	61.2	—
	N/14 H_2SO_4	1	57.2	27.4
	—	0	59.3	3.4
	N/14 NaOH	1	53.8	—
	N/14 NaOH	2	45.1	0.0
	N/14 NaOH	3	36.5	—
	N/14 NaOH	4	27.0	0.0

The change in hydrogen ion concentration of the naturally buffered juice caused by the small amounts of $N/14$ acid and base added was not sufficient to bring about any immediate granulation which could be observed microscopically. This may be judged in the case of the acid by comparison with Fig 4, *a*. In the course of 6 hr., however, the acid increased precipitation while the base decreased it. The increments of acid had less effect on the unprotected juice than those of base, possibly because the juice was more strongly buffered against acid than against base, as already noted, but more probably because the original juice was already within the margin of its precipitation zone and in the liberal time allowed for precipitation the acceleration of the reaction by added acid became relatively unimportant. The sugar-protected juice, however, was so stable that the quantity of acid added became very important. The combination of sugar and dilute alkali prevented all precipitation.

TABLE IX

SUGAR PROTECTION AGAINST "SALTING OUT" OF JUICE SATURATED WITH ZINC SULPHATE AT 0° C.

Variety	Date 1925	Age in days	Mg. N in 10 cc. juice		Per cent nitrogen pptd.	
			Total	Coag.	Juice only	Juice+8% sucrose
Buffum	Aug. 24	19	40.8	18.8	58.4	48.9
Kanred	Aug. 24	19	54.6	25.5	82.1	70.3

Salt concentration, the other factor commonly supposed to be active in frost precipitation, was investigated in one experiment at 0° C. with regard to its relation to sugar protection. The results presented in Table IX show that here again the sugar is effective at least to some extent in stabilizing the proteins and reducing precipitation.

Effect of Adding Acid, Base and Salts on Frost Precipitation

If increased acidity and salt concentration on freezing are important factors responsible for frost precipitation of plant proteins, then it would be expected that the addition of acid or salt to plant juice before exposure to frost should increase the amount of precipitation, while the addition of base should decrease it. That this is the case is shown by the experimental results collected in Table X, and for Series C recorded graphically as part of Fig. 3. The small quantities of the reagents added produced no coagulation before freezing which could be observed microscopically. Their concentration would of course be increased many times by the withdrawal of water as ice. The reaction would probably move in the acid direction on freezing, even in those samples to which base had been added, the system being dominated by the natural acids of the juice.

The effect of acid was the same in all experiments in which the concentration added was sufficient to have any effect at all. It is most easily seen in the results with Minhardi juice in Series C, which have been incorporated in

TABLE X

EFFECT OF ADDING ACID, BASE AND SALTS ON PRECIPITATION OF UNPROTECTED AND SUGAR-PROTECTED JUICE IN 5 HR. AT −7° C.*

Material	Reagent added to 25 cc. juice		Per cent nitrogen pptd.	
	Kind	Quantity	Juice only	Juice+8% sucrose†
A. Fulcaster March 7, 1924, plants 53 days old. N in 10 cc. juice: total, 36.6 mg. coag., 23 4 mg.	*N*/14 HCl	1 cc.	12.1	—
	N/14 NaOH	1 cc.	0.0	—
B. Minhardi May 13, 1924, plants 69 days old. N in 10 cc. juice: total, 54.1 mg. coag., 30.4 mg.	*N*/14 H_2SO_4	2 cc.	41.2	—
	N/14 H_2SO_4	1 cc.	38.8	—
	None	—	30.5	—
	N/14 NaOH	2 cc.	27.9	—
	N/14 NaOH	3 cc.	23.9	—
	NaCl	0.5 gm.	44.6	—
C. Minhardi July 1, 1925, plants 16 days old. N in 10 cc. juice: total, 31.8 mg. coag., 15.4 mg.	*N*/14 H_2SO_4	5.0 cc.	70.1	52.3
	N/14 H_2SO_4	4.0 cc.	66.4	50.6
	N/14 H_2SO_4	3.0 cc.	66.5	46.0
	N/14 H_2SO_4	2.0 cc.	62.3	39.4
	N/14 H_2SO_4	1.0 cc.	61.3	34.3
	N/14 H_2SO_4	0.9 cc.	—	33.4
	N/14 H_2SO_4	0.8 cc.	—	30.5
	N/14 H_2SO_4	0.7 cc.	—	30.6
	N/14 H_2SO_4	0.6 cc.	—	39.2
	N/14 H_2SO_4	0.5 cc.	—	18.8
	N/14 H_2SO_4	0.4 cc.	—	11.6
	N/14 H_2SO_4	0.3 cc.	—	6.1
	N/14 H_2SO_4	0.2 cc.	—	5.7
	N/14 H_2SO_4	0.1 cc.	—	2.8
	None	—	61.3	6.5
	N/14 NaOH	1.0 cc.	55.3	5.0
	N/14 NaOH	2.0 cc.	48.6	5.5
	N/14 NaOH	3.0 cc.	41.9	6.6
	N/14 NaOH	4.0 cc.	34.6	10.8
	N/14 NaOH	5.0 cc.	35.5	22.2
D. Minhardi March 2, 1925, plants 13 days old. N in 10 cc. juice: total, 47.1 mg.	None	—	35.6	—
	NaCl	0.25 gm.	47.4	7.7
Fulcaster March 4, 1925, plants 15 days old. N in 10 cc. juice: total, 40.9 mg. coag., 25.4 mg.	None	—	48.3	—
	NaCl	0.25 gm.	52.7	6.7

**In Series A, exposure was 4 hr. at 0°C. followed by 2 hr. at −21° C*

†In Series F, concentration of sucrose was 4%.

TABLE X—*Continued*

Material	Reagent added to 25 cc. juice		Per cent nitrogen pptd.	
	Kind	Quantity	Juice only	Juice+8% sucrose†
E. Buffum	None	—	65.6	—
Aug. 24, 1925,	*N*/1 Na_2HPO_4	1 cc.	62.7	16.8
plants 19 days old.	*N*/1 NaCl	1 cc.	60.1	15.8
N in 10 cc. juice:	*N*/1 CH_3COONa	1 cc.	59.9	10.5
total, 40.8 mg.	*N*/1 Na_2CO_3	1 cc.	39.5	12.1
coag., 18.8 mg.				
Kanred	None	—	59.1	—
Aug. 24, 1925,	*N*/1 Na_2HPO_4	1 cc.	59.5	13.1
plants 19 days old.	*N*/1 NaCl	1 cc.	56.3	27.3
N in 10 cc. juice:	*N*/1 CH_3COONa	1 cc.	52.1	13.7
total 54.6 mg.	*N*/1 N_2CO_3	1 cc.	27.2	16.8
coag., 25.5 mg.				
F. Kanred	None	—	43.4	36.0
July 21, 1924,				
plants 21 days old.	*N*/14 H_2SO_4	1 cc.	41.4	39.9
N in 10 cc. juice:				
total, 48.4 mg.	NaCl	0.25 gm.	41.5	35.5
coag., 27.5 mg.				
Juice as above,	None	—	80.0	22.8
dialyzed.				
N in 10 cc:	*N*/14 H_2SO_4	2.5 cc.	92.0	—
total, 20.5 mg.				
coag., 18.1 mg.				
Kanred	None	—	73.0	17.3
July 25, 1925,				
plants 25 days old,	*N*/14 H_2SO_4	0.8 cc.	87.8	86.7
juice dialyzed.				
N in 10 cc.:	NaCl	0.25 gm.	63.4	44.0
total, 13.9 mg.	NaCl	0.50 gm.	51.8	—
coag., 11.5 mg.	NaCl	0.75 gm.	45.5	—

**In Series A, exposure was 4 hr. at 0°C. followed by 2 hr. at −21° C.*

†In Series F, concentration of sucrose was 4%.

Fig. 3. They follow very closely the results with Fulcaster juice obtained without freezing, which are included in the same figure and have been discussed above. There is this difference, however, that in the Minhardi samples the addition of base was carried to a higher concentration, and shows signs of having gone beyond the limit of the zone in which its action is protective.

Experiments on the effect of adding common salt occur in Series B, D, E and and F. The last series will be discussed below. In the other three, it will be seen that when the added concentration was 1% (0.25 gm. to 25 cc.) or over, the frost precipitation was increased. In Series E, three other salts, with buffer properties, were added in equimolar concentrations. The percentage thus varied with the molecular weights, being in the case of the common salt about 0.23%. This was probably too small to exercise any "salting out" effect, even when concentrated by freezing. In effect on precipitation, the

four salts fell in the same order with both Buffum and Kanred juice, and the order was definitely related to their buffer properties. The acid sodium phosphate reduced the precipitation only slightly if at all, while the sodium carbonate, an alkaline buffer, reduced it very greatly.

Series F includes three experiments, in the last two of which the juice was dialyzed free from electrolytes. The purpose was to examine separately the salting-out and acid-precipitation effects, uncomplicated by the action of acids and salts naturally present in the juice. Two features of the results are especially noteworthy.

First, the precipitation of the dialyzed juice was greater than that of the original juice, when no sugar had been added to either. The comparison here is best made on the basis of coagulable protein, since the non-coagulable nitrogen was largely removed by dialysis. In Kanred collected July 21, the frost precipitation was equal to 76% of the coagulable protein in the original juice and 90% in the dialyzed juice. In the collection of July 25, the precipitation was equal to 88% of the same fraction in the dialyzed juice. Thus it appears that the practically complete removal of natural electrolytes increased frost precipitation, and we must perhaps ascribe the whole of the precipitation in the dialyzed juice to the direct dehydration of the proteins by abstraction of water as ice.

The addition of a small amount of dilute acid to the dialyzed juice increased precipitation to more than the equivalent of the total coagulable protein. This was observed before, in titrating fresh juice with acid and alkali (Table VII), when it was concluded that some of the non-coagulable proteins were precipitated by acid.

The second striking feature of the experiments of Series F is the protection against frost precipitation of dialyzed juice afforded by added salt. This increased with concentration within the 1 to 3% range used. Such a result was unexpected at the time, though it was encountered again later (11), when it was found that the removal of crystalloids from plant-juice by dialysis made the proteins very unstable, and that their stability could be partly though not completely preserved by dialyzing against a 1% solution of common salt.

Taking Table X as a whole, no more significant generalization emerges than the value of sugar in stabilizing the proteins of the juice under all experimental conditions. When the reagent, like dilute acid, is precipitative, sugar is inhibitive; when the reagent, like dilute alkali, alkaline buffers, or in the dialyzed juice, salt, is protective, the action of the sugar is additive.

Irreversibility of Precipitation and Types of Coagulum

The degree of irreversibility of the precipitation caused by frost or added reagents *in vitro* may perhaps be taken as some indication of the probable destructiveness of the same kind of reaction in the living plant. A few simple experiments were done to see whether the various coagula could be redispersed.

A tube of Minhardi juice was exposed to $-7°$ C. for 5 hr. The juice was then thawed and shaken vigorously for several minutes. Microscopic examination

KEY TO FIG. 4

Minhardi juice
First visible granulation
20 cc. juice
7 cc. $N/14$ H_2SO_4

a

Minhardi juice
Flocculation
20 cc. juice
12 cc. $N/14$ H_2SO_4

b

Minhardi juice
Maximum precipitation
20 cc. juice
15 cc. $N/14$ H_2SO_4

c

Minhardi juice
Beginning of dispersion
20 cc. juice
17 cc. $N/14$ H_2SO_4

d

Minhardi juice
Increasing dispersion
20 cc. juice
30 cc. $N/14$ H_2SO_4

e

Minhardi juice
After neutralization
20 cc. juice
2.5 cc. $N/5$ H_2SO_4
2.5 cc. $N/5$ NaOH

f

Fulcaster juice
Maximum precipitation
20 cc. juice
2 cc. $N/5$ H_2SO_4

g

Fulcaster juice
After neutralization
20 cc. juice
2 cc. $N/5$ H_2SO_4
2 cc. $N/5$ NaOH

h

Fulcaster juice
Sugar-protected
20 cc. juice
1.6 gm. sucrose
2 cc. $N/5$ H_2SO_4

i

Minhardi juice
Frost precipitation
5 hr. at $-7°$ C.

j

Minhardi juice
Sugar-protected
5 hr. at $-7°$ C.

k

Minhardi juice
Heat coagulation
30 min. at 97.5° C.

l

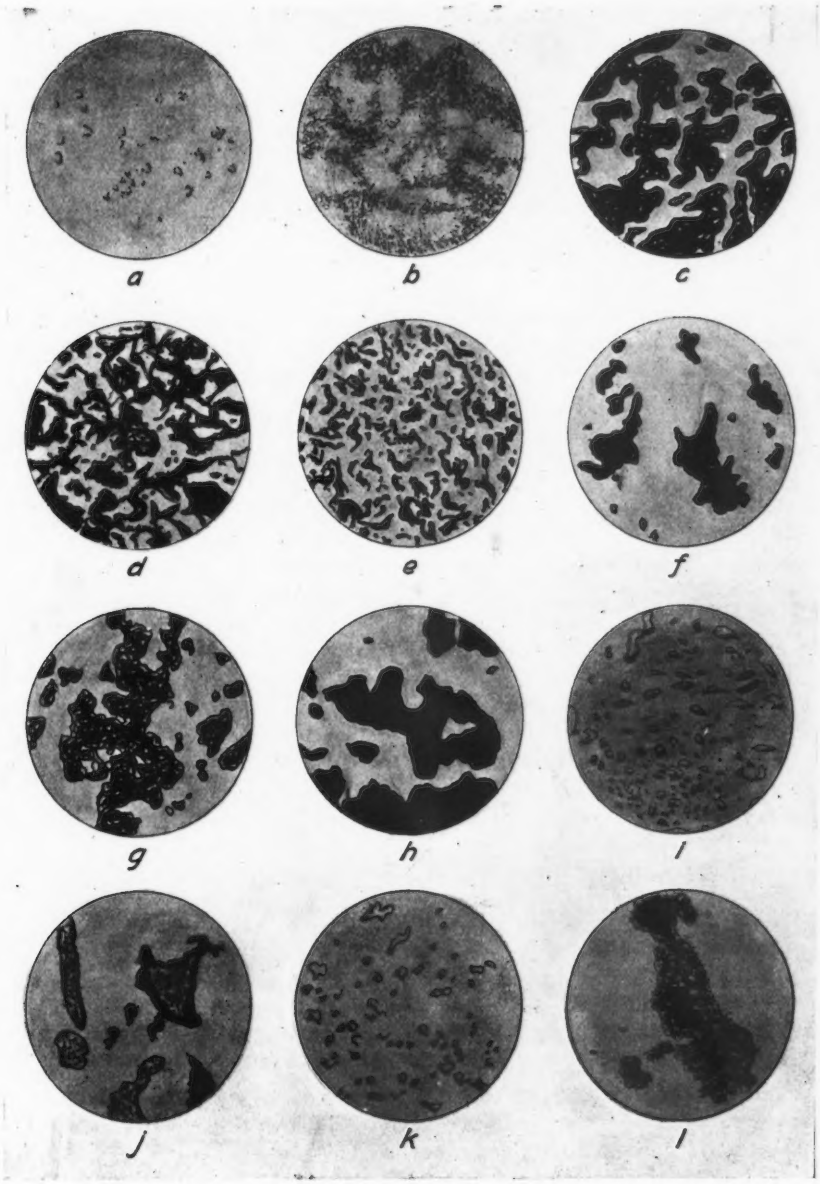

FIG. 4. *Tracings of micro-projections of 1-mm. portions of smears caused by drops of juice flowing across glass slides held at an angle of 45°.*

of the juice before and after shaking showed that instead of redissolving, the small particles of coagulum had flocculated into larger masses, the effect being analogous to the formation of butter by churning. That this shaking even incorporated some of the finer particles which would otherwise have remained in suspension upon centrifuging, is shown by the fact that the supernatant fluid from shaken juice contained 0.8 mg. of nitrogen less per 10 cc. than that of the unshaken control sample.

When small quantities of acid or base were added to wheat-leaf juice a precipitate was obtained when the acidity of the solution reached about pH 5.1 or pH 7.3 respectively. In order to see whether this precipitation was reversible, samples were neutralized by the addition of an equivalent quantity of base or acid. Instead, however, of dissolving the particles this procedure invariably resulted in the formation of a more pronounced, denser precipitate. There seemed to be a further abstraction of water from the particles, causing them to shrink and take on the appearance of a firmer texture. This behavior is illustrated in Fig. 4, *c* and *f*, *g* and *h*. That this denser precipitate was not due to the salt formed by the chemical combination of the acid and base, was shown by the lack of any trace of precipitate when an amount of neutral salt solution, equal to that formed by the combination of the two reagents, was added to the juice. In fact when double this amount of salt was added no precipitation occurred.

When the smear method of examining the precipitation points of juice protein was first used, it was noted that the coagula produced by various agencies were very different.

At the acid precipitation point, the particles of the coagulum formed are always granular in structure and honeycombed with holes (Fig. 4, *c* and *g*). They are light green in color and very irregular in outline. If this coagulum is neutralized by a base, the particles immediately become more compact and regular in outline and take on a denser appearance (Fig. 4, *f* and *h*).

At the basic precipitation point the coagulated particles are dark green in color and, unlike the large, irregular, granular particles of the acid coagulum, they are small, somewhat spherical in shape, and of a more even-textured appearance. If acid is added these spherical particles at once aggregate and form a coagulum similar to that of the neutralized acid coagulum.

If sugar is first added to the juice and then acid, a coagulum is formed which is very similar in general character to, but with larger and more numerous particles than, the basic coagulum just discussed (Fig. 4, *i*). If sugar is added, then a base, the particles are scarcely visible.

The particles of the coagulum formed by heat are large, granular in structure but almost colorless (Fig. 4, *l*). In general shape and size they resemble the acid-coagulated particles, but are a little more compact than the latter.

Unlike any of the coagula so far described, that formed by exposure to frost appears to consist of thin flakes, often roughly rectangular in outline, and generally rolling up at the edges (Fig. 4, *j*). The structure of the flakes is homogeneous rather than granular. The sugar-protected frozen juice shows a character similar to that of the basic coagulum (Fig. 4, *k*).

Another feature noticed during the course of the work was that the heat coagulum of unprotected juice which had just been frozen, contains white curds to the extent of about a fourth of its volume. This is observed only when heat coagulation is preceded by exposure to frost, and suggests that freezing brings about a separation of some of the pigments and proteins.

Discussion

The nature of the experiments described in this paper precludes a high degree of accuracy in the results. Plants grown under uncontrolled conditions are highly variable in their properties, and some of the laboratory manipulations required are not amenable to strict precision. For example, precipitation was not always clean cut, and before sampling it was necessary to decant the whole of the fluid portion after centrifuging, since pipetting from various depths in the test tube would yield fluid of different concentrations. There is, nevertheless, sufficient consistency in the results to justify certain deductions.

It seems clear that both "salting out" and acid precipitation play a part in frost precipitation, and that sugar is an important factor in protecting the protoplasmic proteins from disorganization by these agencies. It cannot be concluded, however, that salt and acid are the fundamental factors in precipitation, since this took place to an even greater extent in dialyzed juice, and under these conditions the addition of a small concentration of salt exerted a protective influence.

It seems probable that dehydration of the proteins is the basic cause of precipitation, and that the agency is incidental rather than fundamental. The degrees of precipitation brought about by freezing, by titration with acid, and by saturation with zinc sulphate, were in general quite similar, and may simply indicate that all these agencies, when given full scope, are about equally effective in dehydrating the protein particles. The withdrawal of water as ice may well be in ordinary circumstances the dominating factor, but the experimental results leave no doubt that acidity and salt concentration are important modifying influences.

The possible effect of the direct pressure developed in the system by freezing must not be overlooked. Maximov (6), in his recent review of the principal theories concerning frost-killing of plant tissue, reaffirms the view he first put forward in 1914, that the reason is the mechanical injury of the protoplasm caused by the compression of the cells by ice crystals which accumulate in the intercellular spaces. That protoplasm may be coagulated by pressure is well known to physiologists. The very labile cell proteins dispersed in press-juice would undoubtedly be subjected to pressure when the system was frozen. Under this influence the frost coagulum might take on the flaky structure which distinguished it from all others, much as the gluten in bread dough takes on a honeycomb structure under the influence of internal gas pressure. Pressure may denature the proteins, and contribute to the irreversibility of the precipitation.

The term precipitation has been used in this paper in the general sense of

the separation of the solid and liquid phases. When the separation takes place at the isoelectric point, as in titration with acid, initially it may be true precipitation, but it is evidently followed by denaturation, since the process is irreversible. Salting out may follow somewhat the same course. When heat is the agent, undoubtedly the process is coagulation. The true character of frost precipitation may be open to question. That which results from dehydration by ice crystallization would seem to be properly classified as coagulation, being irreversible, and must be the condition in dialyzed juice. In ordinary circumstances, frost precipitation may occur at the isoelectric point, and may then be a combination of true precipitation and true coagulation.

The mechanism of sugar protection is still obscure. Maximov (6), after reviewing the work of various authors, concludes that the possibility of a purely chemical activity of sugars is not excluded, but that the action is more probably physico-chemical, based on the molar concentration of the sugar, that is, on the number of molecules present in the solution. He further affirms that the main point in this activity is the retention of part of the water in an unfrozen state, since he believes that it is the quantity of ice formed during freezing which conditions the death of plant cells.

Our results have produced objections to both of Maximov's conclusions. If the activity is proportional to molar concentration, this must be true only within fairly restricted limits, since approximately maximum protection is afforded by 8% of sucrose. On this basis, maximum protection with dextrose should be reached at a little over 4%. The results in Table I, Series D, do show in three cases out of four a slight superiority of 4% dextrose over 4% sucrose. This lends color to the view that at that concentration dextrose is exerting its maximum protective activity while sucrose is not. The point should be examined in further experiments. Maximov's belief that the activity of sugar depends chiefly on the retention of part of the water in an unfrozen state, meets a difficulty in the fact that protection is equally demonstrated when no ice at all is formed, as when the juice is precipitated by titration or salting out, or simply allowed to precipitate spontaneously on standing at 0° C.

The added sugar had no measurable effect on the hydrogen ion concentration of the juice, as influenced by standing at 0° C. or by freezing and thawing. It is true that we did not press out the fluid from the ice crystals for separate measurements, as Harvey did (3), and we could not determine the pH after the juice solidified at about $-2°$ C. But under conditions which precipitated unprotected juice without freezing, the sugar-protected samples at the same pH did not precipitate.

Sucrose in high concentrations, such as should result when an 8% solution freezes, is usually regarded as a dehydrating agent, and might be expected to increase salting out. Sucrose and dextrose theoretically should differ in this respect, the former being hydrated in solution and the latter probably not. Comparative experiments on this point have not been carried out with the two sugars, but we have seen that when sucrose was added to the juice, the amount of salting out was not increased but, on the contrary, decreased.

Some chemical effect of added sugar will be indicated in the next paper of this series, but a complete explanation of sugar protection must await further investigation. It seems possible that additional light may be found in the colloidal phenomena of adsorption and viscosity.

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STUDIES IN THE VARIABILITY OF TUBERCLE BACILLI. II. CORRELATION OF COLONY STRUCTURE, ACID AGGLUTINATION AND VIRULENCE[1]

By Guilford B. Reed[2] and Christine E. Rice[3]

Abstract

Twenty-eight cultures of tubercle bacilli including human, bovine and avian forms from widely differing sources have been compared as to colony structure, habit of growth in fluid media, acid agglutination and virulence for animals.

It was found that the recently isolated highly virulent cultures and all the cultures with a long history of high virulence grew on solid media in S colony form; on fluid media, in the case of bovine and human types, as a continuous veil-like pellicle, in the avian types as a diffuse suspension; all were agglutinated only at a high acidity. All the avirulent or low virulent cultures or those with a history of loss of virulence now grow on solid media in R colonies; in fluid media as a heavy pellicle tending to separate into discrete islands; and are agglutinated at a relatively low acidity.

In the first paper in this series, Reed and Rice (20), it was shown that under appropriate cultural conditions a rapid-growing strain of bovine tubercle bacilli might be separated, on the basis of colony structure, into two types, R and S, which exhibit some degree of stability or true breeding qualities. Under similar conditions several other acid-fast species, particularly *Mycob. leprae* and *Mycob. phlei* (19) were separated into two definite types, R and S, and several intermediate types. These results were shown to be in conformity with the earlier work of Petroff and associates on the tubercle bacilli.

Petroff (13, 14, 15, 16) demonstrated that certain cultures of human, bovine and avian tubercle bacilli may be dissociated by suitable cultural procedure into R and S colony types. A culture of avian organisms which he had had under cultivation for many years was dissociated into an S type, highly virulent for chickens, and an avirulent R type. The differentiation of these types has since been confirmed by Kahn and Schwarzkopf (6) and additional distinguishing characteristics added. The Saranac strain of human bacilli, H37, which has frequently been used in experimental work, generally characterized by high virulence for guinea pigs, has occasionally failed to produce progressive disease, a condition which Petroff has shown in certain instances to be the result of dissociation from S to R. A bovine strain which had long been under cultivation was successfully dissociated into a virulent S type and an avirulent R type. In the same manner Petroff has separated R and S types from several cultures of B.C.G.

As a further phase of a general study of these phenomena a considerable group of cultures of tubercle bacilli have been examined as to colony structure,

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Contribution from the Department of Bacteriology, Queen's University, Kingston. This work has been carried on with the co-operation and the financial assistance of the Associate Committee on Tuberculosis of the National Research Council. This paper forms one section of a thesis submitted by Dr. Rice to the University of Toronto in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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acid agglutination and virulence for laboratory animals.. In this particular investigation we have not been concerned primarily with the experimental production or observation of the progress of dissociation but with examination and correlation of these three characteristics in a considerable group of cultures. For the most part these are strains which have been under cultivation for a long time and several are cultures which have been extensively used in experimental studies of tuberculosis.

The results reported in this paper are largely based upon a study of colonies appearing on the first series of plate cultures made from the original tubes taken from our own culture collection or as received from other collections. Petroff's gentian violet-egg was used as a plating medium. This was made according to Petroff's (12) original formula except that only one-half the specified gentian violet was added. The reaction was accurately adjusted with the aid of the hydrogen electrode to pH 7.8 before sterilization and the sterilization was carried out at 100° C. In some instances Petri dishes were used, sealed with paraffin except for a small air vent; in the majority of cases Petroff's excellent modification of Kolle flasks, now made in Pyrex glass, were used. These permit examination of colonies with a 25 to 50-diameters binocular microscope almost as readily as when the organisms are grown in Petri plates.

As a further measure of the characteristics of the several cultures, suspensions were subjected to acid agglutination. In this procedure the growth was removed from the surface of the media with a spatula, ground in either a mortar or a small ball mill*, with the addition of distilled water, drop by drop, to form an even suspension. After the organisms had been washed three times by centrifuging in distilled water, the washed suspension was added to a series of phosphate buffer solutions ranging from pH 2.0 to 5.0. The resulting agglutination was read after incubation for two hours at 40° C. As has been observed in many such studies, agglutination occurred at a particular pH zone. In the brief statement of results with many cultures, in this paper, the highest pH to produce a definite agglutination is taken as the end-point. In the case of a culture which is completely agglutinated from pH 2.0 to 3.2 the latter figure is recorded as the agglutination point.

Acid agglutination was resorted to as a means of differentiation in one of the earliest studies of bacterial dissociation; DeKruif (3) separated the organisms of rabbit septicemia into two types on the basis of colony structure and demonstrated a correlation between this characteristic and both virulence for animals and the point of acid agglutination. Since this work of DeKruif's much has been done on the potential difference of bacteria in many aspects. Most significant in this connection is the demonstration of Kahn and

**This small ball mill has been found very useful in preparing suspensions of tubercle bacilli for various purposes. Ordinary 125-cc. Florence Pyrex flasks into which half a dozen glass beads or better, 5-mm. steel balls as used in bearings, are added, constitute the grinding chamber. This is clamped to the centre of a horizontally revolving wheel and turned at a rate of one to ten revolutions per second. A number of these flasks are prepared, plugged with cotton, and sterilized. As required the dry pellicle or other growth mass is added, the flask clamped to the wheel and grinding commenced. Fluid may be added with a pipette as required to give an even suspension. It is no more efficient as a grinder than a mortar but it does reduce contamination to a minimum.*

Schwarzkopf (6) that Petroff's R and S strains of avian tubercle bacilli show a substantial difference in electrophoretic potential. It is quite possible that we should have been better advised to follow such a practice, or McCutcheon, Mudd, Strumie and Lucké's (9) procedure of determining isoelectric points, rather than to determine the probably similar characteristics of the organisms by acid agglutination. Later and more detailed studies with a small number of cultures bear out the suggestion. However it will be observed that a definite correlation was found in a considerable group of cultures between colony structure, virulence for animals and acid agglutination. The method appears therefore to have been sufficiently accurate for the distinctions which are made in this paper.

Human Types

Human—H37

A culture of the Saranac Lake strain of virulent human type was sent to us by Dr. Petroff. The first culture on gentian violet-egg media plates produced only characteristic S colonies. Young cultures on Proskauer and Beck's fluid grew as a thin continuous veil which gradually thickened as the culture matured. Suspensions were agglutinated at pH 3.2. This apparently represents the characteristic S, virulent, human type.

Human—Strains Freshly Isolated from Sputum

Six strains have recently been isolated from the sputum of active pulmonary cases. The initial cultures consisted entirely, or largely, of definite S types. Morning samples of sputum were mixed with equal volumes of three per cent sodium hydroxide and allowed to stand with occasional shaking for two hours. Hydrochloric acid was then added to the first yellow tinge of phenol red, about pH 7.2. After centrifuging 15-cc. portions of the fluid, the supernatent 12 to 14 cc. was removed and discarded and Petroff's gentian violet-egg media inoculated from the surface of the remaining 1 to 3 cc. of fluid in such a way as to avoid the packed sediment at the bottom of the centrifuge tubes.

The initial cultures prepared in this manner and incubated for six to eight weeks exhibited very similar S colony types. Plate I, 1 to 6, are photomicrographs made from these cultures and indicate the type of colony found in the six samples. The colonies, with their granular surfaces, raised central areas sloping gradually to a very thin margin and irregular outline, closely resemble the S type of the H37 culture just described. In those cases where a good growth was obtained on a number of plates, so that thirty to fifty thousand colonies could be compared, a few R-like types were found. A study of these types from a number of typical and atypical cases is now in progress and will be reported on in the near future.

All these strains with the S colony structure showed acid agglutination at approximately the same pH level and in sharp contrast to the R human types mentioned in this paper. The results are shown in Table I. The same six strains freshly isolated from sputum produced progressive tuberculosis in guinea pigs.

TABLE I

SUMMARY OF THE COLONY STRUCTURE, ACID AGGLUTINATION AND VIRULENCE OF A GROUP OF 28 CULTURES OF TUBERCLE BACILLI

Culture	Colony structure	Acid agglutination, pH	Virulence
Human			
H 37	S	3.2	+
Sputum 1	S	3.0	+
2	S	3.0	+
3	S	3.2	+
4	S	3.2	+
5	S	3.0	+
Koch	S	3.2	+
H 13 S	S	3.3	+
H 13 R	R	3.9	−
Ceylon	R	3.9	−
Koch-Raw	R	3.8	−
54 S	S	3.2	?
54 R	R	4.2	−
Upt	R	?	−
Bovine			
Vallée S	S	2.8	+
Vallée R	R	3.8	?
56	S	2.8	+
Calmette-Raw	R	4.2	−
Brown	R	4.0	−
599	R		−
Avian			
Petroff S	S	2.8	+
Petroff R	R	3.6	−
823	S	2.8	+
807	S	3.4	+
116	R	3.8	−
118	R	4.0	−
Bang	R—S	4.0	−
120	R—S	4.0	−

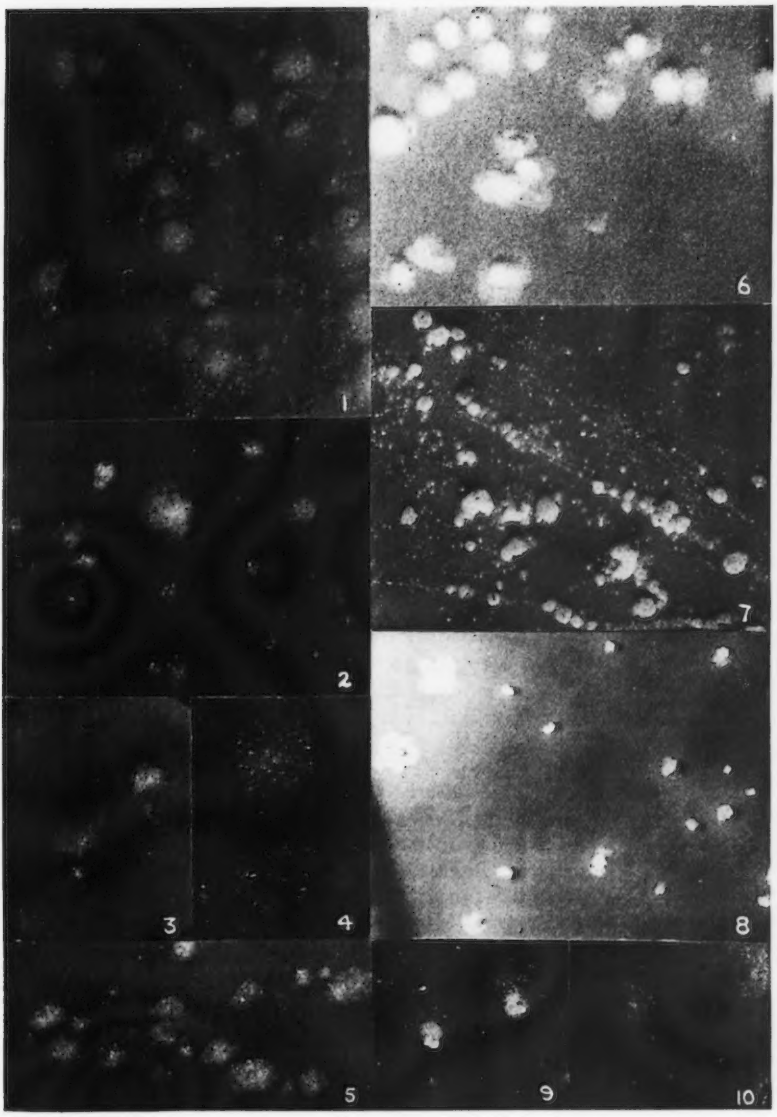
Human—Koch Strain

This culture was obtained from the National Type Culture Collection, London, in 1926. According to the Collection List it is Koch's original strain, although we have not obtained any further history of the culture. During the last five years it has been maintained on glycerol-egg media.

Plated on gentian violet-egg media irregular, granular and spreading S type colonies were produced, Plate I, 7 and 8, Agglutination occurs at pH 3.2. Progressive disease is produced in guinea pigs. This culture then appears to have maintained S characteristics through many years of laboratory cultivation.

Human—H13

This culture is used by Mrs. F. Maltaner of the New York State Department of Health, Albany, in the preparation of antigens for the routine complement fixation reaction for tuberculosis. It has had a long history of high virulence since its isolation in 1909 or 1910 by Krumwiede, late of the New York City Research Laboratories, from a guinea pig injected with tubercular sputum.



Photographs of colonies of tubercle bacilli grown on gentian violet-egg media. FIG. 1-6. *Colonies from the first culture from six samples of tubercular sputum; four to six weeks growth.* FIG. 7 AND 8. *S type colonies, Koch, human strain.* FIG. 9 AND 10. *S type, No. 13, human.*

When received in July 1930 and plated on gentian violet-egg media it produced granular and irregular S colonies (Plate I, 9 and 10). On Proskauer and Beck's fluid it grew as a thin continuous veil which gradually thickened with maturity. Suspensions were agglutinated at pH 3.2 to 3.4. As indicated also by the history of its behavior in guinea pigs it appears to have maintained its S form for some twenty years in laboratory cultures.

Since it came into this laboratory it has been grown for several generations on Sauton's fluid adjusted to an initial reaction of pH 6.2 to 6.4. Successive cultures have shown less of the spreading continuous veil-like pellicle and more heavily wrinkled areas tending to separate into floating islands (Plate III, 33). Gentian violet-egg media plates now show few of the S type of colonies, just noted, but a majority of perfectly smooth, waxy, regular, mound shaped, or R, colonies. These in suspension are agglutinated at pH 3.8 to 4.0 and fail to produce progressive disease in guinea pigs in doses up to 5 mg. This evidently represents an S to R dissociation during less than a year's cultivation on acid Sauton's fluid.

Human—Ceylon

A culture labelled in this manner was obtained from the National Type Culture Collection, London, in 1931 and was said to have been isolated in Ceylon three years ago. Plated on gentian violet-egg media, colonies developed which for the most part were definitely R in type (Plate II, 11) although a very few showed a granular spreading margin. Suspensions are agglutinated at pH 3.8 to 4.0. Guinea pigs injected with doses up to 5 mg. failed to develop progressive disease. Whatever the original history of this culture may have been it is clearly now avirulent and primarily R in type. The spreading granular margins which have grown out from occasional colonies suggest that an S form might readily be separated.

Human—Nathan Raw-Koch

This presumably is one of Koch's original strains which was sent to Dr. Nathan Raw in 1904 and was received from the National Type Culture Collection, London, in 1930. The culture obtained from London was plated directly on gentian violet-egg media. The colonies were all perfectly smooth waxy mounds without any indication of granular forms or spreading colony margins. This colony form strangely suggests an avian type (Plate II, 13). On liquid media a tendency to diffuse growth also suggests an avian form. Agglutination or emulsions occurred at pH 3.8. Guinea pigs were not infected by doses up to 5 mg.

After this examination was made there came to the attention of the authors a paper by Wilson (24), dealing apparently with the same culture, in which he concludes that this is an avian strain masquerading under the wrong name.

Human—54

According to the history of this culture it was brought to the United States in 1888 from Koch's laboratory by Vaughan. In its 108th transfer it exhibited less than its original virulence for guinea pigs. We obtained it from the New

York State Department of Health, Albany, in 1930. In its present form the culture grows at an entirely atypical rate and degree of luxuriance, reaching a maximum growth on favorable solid or liquid media in four to seven days. When plated on gentian violet-egg media the characteristic R colonies which develop (Plate II, 12) closely resemble those from a rapid-growing bovine strain, described in detail in the first paper of this series (20). In contrast to the several S-type suspensions which, as just indicated, are agglutinated at pH 3.0 to 3.2, suspensions of this R culture were agglutinated at pH 4.2. In guinea pigs 5-mg. doses produced only very slight local lesions which were resorbed in two to three weeks.

After the culture had been grown for a number of generations in large flasks of Proskauer and Beck's synthetic fluid medium, well buffered to pH 7.8 to 8.0, a few thin granular patches of pellicle appeared in contrast to the typical massive waxy folded growth. On fishing these granular pellicle fragments and transferring to gentian violet-egg plates, colonies appeared which were quite definitely S in form (Plate II, 12A). Suspensions were agglutinated at pH 3.4 as S. These new S forms were highly unstable and very readily reverted to the R form.

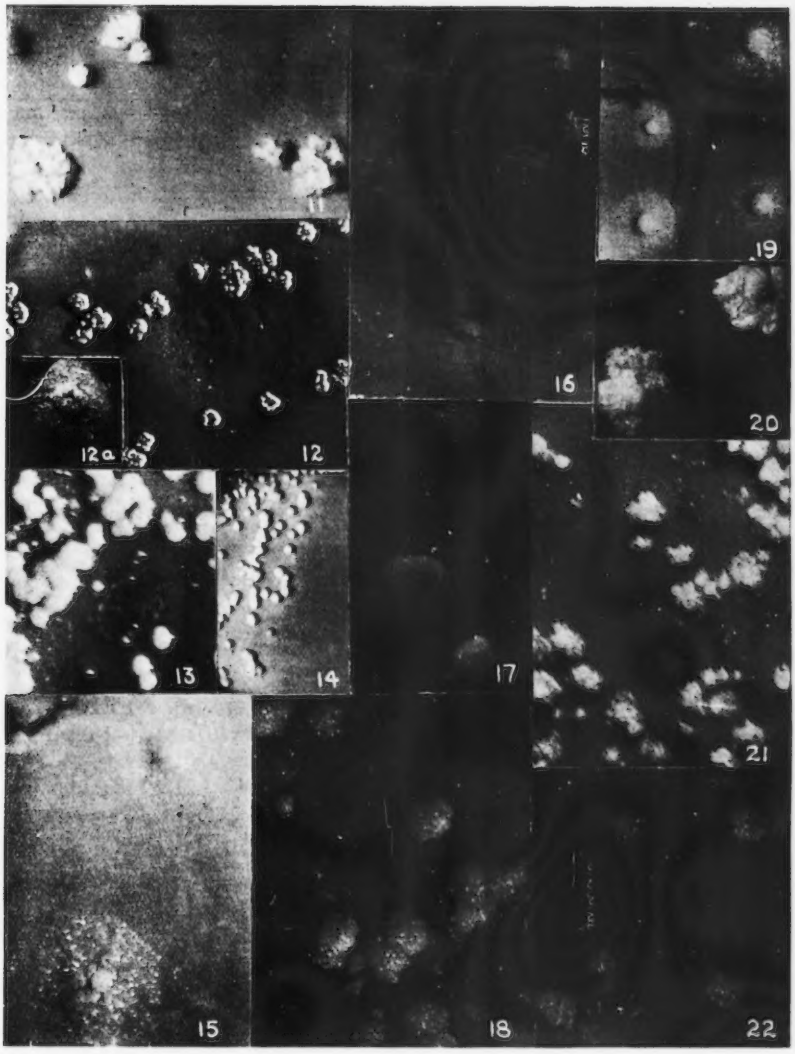
Human—Upt

This culture was isolated by one of us in 1922 from the lung of a girl who died of miliary tuberculosis. No examination of the colonies was made at that time but the culture was used for the next two or three years in class work and for the experimental production of tuberculosis in guinea pigs. After six years of cultivation on glycerol-egg media it was plated on gentian violet-egg. The colonies which appeared and have continued to appear are perfectly regular smooth waxy hemispherical mounds and suggest, as in the case of the Nathan Raw-Koch strain, an avian form (Plate II, 14). Suspensions are agglutinated at pH 4.0 to 4.4. Doses of 5 mg. produce in guinea pigs either no infection or only slight local lesions. It is probable that this is not the culture originally isolated.

Bovine Types

Bovine—Vallée

A culture of the familiar Vallée strain was sent to the authors by Dr. Watson of the Canadian Department of Agriculture, and was carried in our laboratory for about two years on glycerol-potato before the present examination was made. When first plated on gentian violet-egg media the majority of colonies consisted of granular spreading structures with a slightly raised centre and a very thin irregular margin (Plate II, 15 and 16). These colonies closely resembled the S form previously described (20) and were in every way similar to the bovine S described by Petroff (17). The S types in subcultures on gentian violet-egg media have persisted without apparent change for several generations. In Proskauer and Beck's buffered alkaline fluid they grow as a continuous thin pellicle which gradually becomes thickened and folded as the culture matures. Suspensions were agglutinated at pH 2.8. Progressive tuberculosis was produced in guinea pigs. This apparently represents the characteristic S, virulent, bovine form.



Photographs of colonies of tubercle bacilli grown on gentian violet-egg media. FIG. 11. *R type, Ceylon, human.* FIG. 12. *R type, No. 54, human.* FIG. 13 AND 14. *Avian-like colonies with a questionable history of human strains (see text).* FIG. 15 AND 16. *S type, Vallee bovine strain.* FIG. 17 AND 18. *R type, Vallee bovine.* FIG. 19 AND 20. *S type, No. 56, bovine.* FIG. 21. *R type, Calmette, N. Raw, bovine.* FIG. 22. *R type, Brown, bovine.*

The original plates made of the Vallée strain, after the authors had carried the culture for two years on potato media, in addition to the S forms just mentioned exhibited a very small proportion of R colony types, 1 R to 5000 or 10,000 S. These R colony forms grew initially as regular hemispherical mounds with a slightly pebbled surface, regular outline and rising abruptly from the surface of the medium (Plate II, 17 and 18). Later they became umbilicated, or folded, similar to those described in detail for the R bovine 599 (20). In Proskauer and Beck's fluid, growth occurred in island-like masses rather than in the continuous veil form of the S. Suspensions were agglutinated at pH 3.8. Doses up to 5 mg. produced conspicuous local lesions but failed to produce a progressive disease in guinea pigs.

It seems apparent that this bovine strain which has long maintained a high degree of virulence underwent a partial S to R dissociation with accompanying loss of virulence previous to or during our two years of cultivation on potato media.

Bovine—561

This culture is probably one of those described by Ravenal (18), and has a long history of high virulence. We received it from the New York State Department of Health in 1930. Plates grown from the original culture received exhibited only the typical granular spreading S colonies, (Plate II, 19 and 20), similar to those of the S form of the Vallée strain. Cultures on Proskauer and Beck's fluid develop as a thin continuous veil gradually thickening into a folded pellicle. Small doses in guinea pigs produced progressive tuberculosis and death in three to five weeks. Suspensions were agglutinated at pH 2.8.

Bovine—Calmette-Nathan Raw

A culture of unknown history with this designation was received from the National Type Culture Collection in 1930. The original plates exhibited for the most part smooth hemispheres or waxy folded R colonies with very few showing granular spreading margins (Plate II, 21). Suspensions were agglutinated at pH 4.2. Doses up to 5 mg. failed to produce progressive disease in guinea pigs. This is evidently a culture principally R but the spreading colony margins, although few, suggest that an S form might be readily developed.

Bovine—Brown

Dr. Caulfield and Dr. Brown of the Connaught Laboratories, Toronto, very kindly supplied the authors with a culture of bovine origin. This culture, they state, exhibited a fairly high degree of virulence when freshly isolated but after several years of laboratory cultivation the virulence has been so far lost that it produces no demonstrable lesions in guinea pigs though the guinea pigs become sensitive to tuberculin. The first culture received from Dr. Brown, plated on gentian violet-egg media, produced characteristic R colonies rising abruptly from regular outlines at the surface of the medium to form slightly pebbled hemispherical colonies indistinguishable from the previously described R Vallée (Plate II, 22). Agglutination of emulsions occurred at pH 4.0.

Bovine—B.C.G.

Several cultures of B.C.G. have been under observation for a number of

years. The typical culture as noted by Petroff and Steenken (17), Begbie (1), Kraus (7), Lange (8), Gerlach (4), Tzeknovitzer (21) and others when plated on gentian violet-egg media produces colonies with a smooth waxy or slightly pebbled surface and regular outline on the surface of the medium though fully mature colonies frequently become considerably folded—the typical R colony form (Plate III, 23 and 24). Growths of this form have been repeatedly obtained. The definite R colonies when injected into guinea pigs even in large doses have produced only localized lesions. Suspensions are agglutinated at pH 4.0, a pH level, as the previous results indicate, characteristic of R forms. Detailed data concerning S forms which have been separated from several B.C.G. cultures will be presented in a separate paper.

Bovine—599

In the first paper of this series (20), it was shown that a rapid-growing bovine strain consisted entirely of R colony forms, but that continued growth on alkaline fluid media resulted in the separation of somewhat atypical S forms.

Avian Cultures

Petroff's Avian R and S

Petroff (17) reported that a strain of avian organisms which he had had under cultivation for a number of years had been dissociated into R and S types. The S he found highly virulent for chickens and the R avirulent. Through the kindness of Dr. Petroff the authors received these dissociated R and S forms more than a year ago. Cultivation on a gentian violet-egg medium has resulted in the formation of colonies similar to those described and figured by Petroff (Plate III, 24 and 25). In 5-mg. doses the S produced progressive disease in pigeons; similar doses of the R failed to produce demonstrable infections.

Kahn and Schwarzkopf (6) working with Petroff's cultures have just shown that the R and S also differ in electrophoretic potentials. We found that R was agglutinated in buffer solutions at pH 3.6 and the S at pH 2.8 which is in approximate agreement with Kahn and Schwarzkopf's electrophoretic potential results.

Six avian strains of unknown history were recently obtained; four in 1928 (823, 807, 116, 118) from the American Type Culture Collection, and two from the Lister Institute Collection, No. 120 in 1926 and Bang in 1930. These were all cultivated for several generations on glycerol-egg before this study was undertaken.

Avian 823 and 807

These two cultures plated on gentian violet-egg media produced perfectly regular, smooth, mound-like colonies (Plate III, 27 and 28), indistinguishable in appearance from the S type of the Petroff culture. In Proskauer and Beck's fluid media the growth was diffuse with very little tendency to produce a pellicle. Suspensions of No. 823 were agglutinated at pH 2.8 and in pigeons 5-mg. doses produced progressive disease. Suspensions of the 807 agglutinated at a much higher pH, 3.4, and though progressive disease was produced in pigeons the development was much slower.

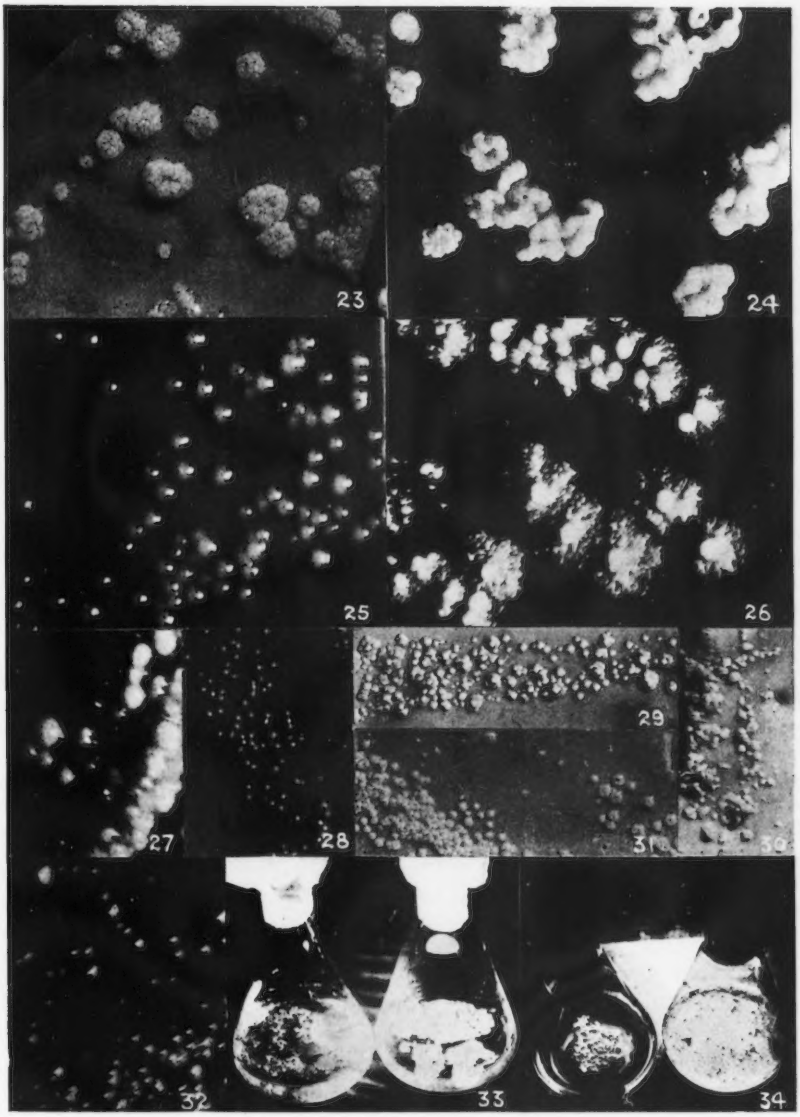


FIG. 23-32. *Photographs of colonies of tubercle bacilli grown on gentian violet-egg media.* FIG. 23 AND 24. *R type, B.C.G., bovine.* FIG. 25. *S type, Petroff's avian strain.* FIG. 26. *R type, Petroff's avian.* FIG. 27 AND 28. *S type, No. 823 and 807, avian.* FIG. 29 AND 30. *R type, No. 116 and 118, avian.* FIG. 31 AND 32. *Intermediate colony types, No. 120 and Bang, avian.* FIG. 33. *Photographs of cultures of human strain No. 13 growing on Proskauer and Beck's fluid; on the left, veil-like S growth with R-like islands; on the right, the thicker R growth.* FIG. 34. *B.C.G.; on the left, the typical R growth; on the right, the veil-like S growth.*

Avian 118 and 116

Gentian violet-egg plates of these two cultures exhibited typical R colonies (Plate III, 29 and 30) indistinguishable from the R forms of Petroff's culture and strikingly unlike Petroff's S forms and those of No. 823 and 807 just described. Both of these in fluid media grew as a surface pellicle in contrast to the diffuse habit of the S and both were agglutinated at a high pH, 3.8 to 4.0. In 5-mg. doses both failed to infect pigeons.

Avian—Bang and 120

The colonies developing from these strains were much less definite than the previously described avian cultures. A few exhibited S characteristics, some were R-like but the majority appeared to be of an indefinite intermediate form (Plate III, 31 and 32). In fluid media growth resembled the R, that is a definite pellicle formed in contrast to the diffuse growth of the S, while suspensions like the R types agglutinated at a high pH, 4.0. In pigeons 5-mg. doses failed to produce demonstrable infection.

Discussion

The data presented in the foregoing sections have been summarized in Table I. It seems evident, as shown in Petroff's work, that there is a definite correlation between colony structure and virulence of human, bovine and avian tubercle bacilli. It has been shown that freshly isolated virulent strains exhibit the S colony structure and at the same time all the cultures examined which have had a long history of high virulence grow on solid media in a similar form. It therefore seems evident that the two characteristics have been maintained in parallel. In contrast all the cultures which exhibit lack of virulence or have a history of loss of virulence grow in R colonies. The instances quoted in this paper, in previous papers in this series and in Petroff's work on change from S to R with accompanying decrease or loss of virulence under experimentally controlled conditions, probably represent the sequence of events in the several cultures with a history of loss of virulence reported on in the previous sections.

Although it has not been studied in the same detail it appears that all the virulent or S human and bovine forms grow in fluid media in the form of a continuous thin and spreading veil which gradually thickens into a heavy folded pellicle, whereas the avirulent or R forms grow as thickened island-like masses. The virulent or S avian forms all appear to grow in fluid media in a diffuse manner in contrast to the avirulent or R forms which grow as a surface pellicle.

The results with acid agglutination show an equally definite correlation between virulence and colony structure. Of the human strains, ten S types were agglutinated at approximately pH 3.2 and five R types at pH 3.8 to 4.2; two bovine S types at pH 3.2 and five bovine R types at pH 4.0 to 4.2; four avian S forms at pH 2.8 to 3.4 and four R forms at pH 3.6 to 4.0. Freud reported that certain stock cultures of tubercle bacilli which had retained virulence for guinea pigs were agglutinated at pH 2.8 to 3.0. Acid agglutina-

tion provides a measure of potential difference (Northrop and DeKruif; Northrop, 11) though probably less definite than direct determination of electrophoretic migration or isoelectric points. The agreement of the acid agglutination results on Petroff's avian R and S forms with Kahn and Schwarzkopf's potential determinations on the same forms and our own potential studies on these and other cultures appear to confirm the theoretical relationship of acid agglutination and potential.

These results appear to be in conformity with the recent demonstration of a relationship between potential difference and virulence in the case of *Pneumococci*, diphtheria baci'li, and other species (5). The early work on acid agglutination, Michealis (10) Beniasch (2), and the more recent work on potential by Falk (5), clearly indicate that potential measurements give no direct indication of virulence, but that within the confines of a species which contains pathogenic and non-pathogenic forms or forms of high and low virulence, the pathogenic or most virulent types exhibit higher potentials than the non-pathogenic or forms of lower virulence. In this instance it seems evident that comparison of acid agglutination of avian and human or avian and bovine tubercle bacilli gives no significant indication of the relative virulence. But it does appear that comparison of the acid agglutination points of a group of cultures of avian or a group of cultures of bovine bacilli or human bacilli does provide an indication of the degree of relative virulence within the group as precise as does a comparison of the colony structure.

The tests of virulence mentioned throughout this paper provide evidence of differences between those cultures which produce definite and rapidly progressive tuberculosis in experimental animals demonstrable in a few weeks, in contrast to those cultures which in rather large doses fail to produce demonstrable progressive disease in this relatively short period. The determinations do not take into consideration the possibility of progressive disease developing after long periods of incubation, as has been so clearly demonstrated to occur in certain instances by Watson (23), nor do they allow for the possibility of progressive disease developing in occasional animals in large groups as described by Uhlenhuth and Seiffert (22) and considered to result from exceptionally low natural immunity. This examination of virulence, therefore, is in no sense germane to the important problem of alteration in virulence of organisms in the body of infected organisms.

This examination, including twenty-eight cultures of tubercle bacilli from widely differing sources, provides evidence that the phenomenon of dissociation and the differentiation of two or more types of the tubercle bacilli, as previously described, is probably of general application and not only of application to special cases, such as B.C.G. cultures. Detailed study of several of the types mentioned is now in progress and will be reported on in the near future.

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STUDIES IN THE VARIABILITY OF TUBERCLE BACILLI. III. INFLUENCE OF X-RAYS UPON DISSOCIATION[1]

By Christine E. Rice[2] and Guilford B. Reed[3]

Abstract

It is shown that X-ray treatment of cultures of a rapid-growing strain of bovine tubercle bacillus showing a tendency to change from R to S promoted the change to a considerable degree. Stable R cultures of the same species are unaffected. Larger doses produce a lethal effect.

Protective colloids such as gelatine or blood serum added to the suspension reduce the effect of the irradiation.

Non-acid-fast bacteria are shown to be much less sensitive to the effects of X-rays, as no change in the rate of dissociation has been observed in actively dissociating cultures with twice the dosage to which tubercle bacilli were exposed.

It has recently been shown that the exposure to X-rays of certain plant and animal tissues, especially tissues undergoing rapid cell division, results in striking modifications in form, structure and physiology. Strangeways and Hopwood (24) working with tissue cultures *in vitro*, Stadler (21) with maize, Goodspeed and Olson (6) with *Nicotiana*, Muller (16, 17) with *Drosophila*, Nadson and Philippov (19) with certain yeasts and molds, have observed irregular mitotic and meiotic division and corresponding irregularities in form. It is apparent from this work that it is at the stage of maximum activity in the cell when the most rapid physical and chemical changes are taking place that X-rays produce the most marked effects. Little effect was noted in cells in a state of nuclear repose.

This work on higher plants and animals suggested that variation or dissociation of bacteria might be similarly influenced by X-rays. Although the observed changes have been associated with reactions in the nucleus, it seemed reasonable to suppose that X-ray radiation might also have an effect upon the reproductive mechanism and heredity of the anucleated bacteria.

Mme. Henri (9) reported an interesting case of what may have been dissociation in *B. anthracis* under the influence of ultra-violet light, a stable form being produced which on inoculation gave rise to a pathological picture different from the normal. Schneider (22) recorded the development after irradiation of a less actively fermenting type of yeast. However, very few such changes have been reported.

Although it is not usually considered that X-rays have much lethal effect upon bacteria, Schepmann and Flecke (23) found that extremely soft rays killed bacteria within a few minutes, while the same dosage of hard rays, which could be obtained only after irradiation for several hours, had only half the effect. Laccassagne and Paulin (13) held that if X-rays exerted any bactericidal effect,

[1] *Manuscript received May 29, 1931.*

Contribution from the Department of Bacteriology, Queen's University, Kingston. A portion of this work was done by Dr. Rice in the Department of Pathology and Bacteriology, University of Toronto and included as one section of a thesis accepted at the University of Toronto in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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this depended upon the individual sensitivity of the organisms and was independent of the size, toxic and chromogenic properties of the organisms. Mayer (14) states that although the degree of chemical change may be in proportion to the amount of energy absorbed, the ensuing physiological effect may bear no such relation, but depends rather upon the character of the rays absorbed.

I. Experimental Method

A rapid-growing strain of *Mycob. tuberculosis bovis* was used. This culture, No. 599, was described in the first paper of this series by Reed and Rice (20). It was shown that under appropriate cultural conditions, the R type, which characterized the culture as first observed, dissociated into definite, reasonably stable S types and also intermediate forms.

For these experiments the cultures were plated on gentian violet-egg media, single colonies selected, suspended in saline, filtered as described previously to remove clumps, and the uniform very dilute suspensions plated directly as controls; after exposure to X-rays they were again plated to test the effect of the irradiation. The X-ray dosage employed in the authors' original experiments was that used by Stadler (21) in his work on maize and was found to be sufficiently effective that it was continued in most of the experiments. Tube filters were employed and the machine used was a Wappler transformer, 12-in. capacity. The dosage was as follows: Volts, 88,000; milliamperes, 3; time, 10 min.; target distance, 10 in.

For the irradiation, filtered suspensions of the bacteria were placed in flat-bottomed crystallizing dishes, 7 cm. in diameter, in which 3 cc. of suspension gave a one-millimetre layer. The suspensions were not stirred during irradiation as the layer of fluid was very thin.

II. The Influence of X-rays on R to S Dissociation

R colonies were selected from a culture series which had been carried for six or more culture generations on gentian violet-egg media and had exhibited only R colonies. For the first experiment three characteristic colonies were selected, the organisms suspended in physiological salt solution, passed through filter paper, and the very dilute suspension plated. A portion of the same filtered suspension was then irradiated and immediately plated. The colony types developing on the two series of plates is shown in Table I, Experiment 1. Experiments 2 to 5, the results of which are also shown in Table I were carried out in exactly the same manner, the only difference being that the organisms were in each experiment taken from different series of cultures though they were all similar in appearance. The results indicated in the table show clearly that the bacteria in the untreated suspensions, with two slight exceptions, bred true to type; whereas the bacteria from the same suspensions after exposure to the dosage of X-rays just described, exhibited in the majority of instances some definite dissociation. Summary of the results in Table I show that of the twenty untreated suspensions tested, two, or 10% of the total, showed enough variation from the normal to be regarded as having undergone some

TABLE I

RESULTS OF X-RAY TREATMENT OF R TYPES

Expt. No.	Source of organisms	Colony forms growing from suspension of organisms		Expt. No.	Source of organisms	Colony forms growing from suspension of organisms	
		Before X-raying	After X-raying			Before X-raying	After X-raying
1	1 R colony	All R	Mostly R, very few S	4	1 R colony	All R	All R
	1 R colony	All R	40% R, 30% S, 30% intermediate		1 R colony	All R	All R
	1 R colony	All R	All R		1 R colony	All R	Mostly R, very few S, very few intermediate
2	1 R colony	All R	Mostly R, very few S, very few intermediate		1 R colony	All R	Mostly R, very few S
	1 R colony	All R	All R	5	1 R colony	All R	All R
3	1 R colony	All R	Mostly R, very few intermediate		1 R colony	All R	98% R, 2% S
	1 R colony	All R	Mostly R, very few intermediate		1 R colony	99% R 1% intermediate	95% R, 5% S
	1 R colony	All R	Mostly R, very few intermediate		1 R colony	All R	All R
	1 R colony	All R	Mostly R, very vew intermediate		1 R colony	All R	All R
	1 R colony	All R	All R		1 R colony	98% R 2% intermediate	90% R, 2% S, 8% intermediate

NOTE:—*Single colonies were emulsified in saline, filtered through paper and plates made before and after X-raying.*

dissociation, while after irradiation 12, or 60%, showed similar or in most cases much more definite dissociation.

The S and intermediate colonies which appeared on the plates made from radiated suspensions exhibited much the same type of variability as similar S colonies which grew from alkaline broth cultures, as discussed in the previous paper concerning this organism (20). An S colony arising from a series consistently R for a considerable previous period generally produced, when suspended in fluid and plated on gentian violet-egg, from 10 to 90% S and the balance intermediate or R types. Repeated fishing and replating of S forms generally increased the percentage although as previously shown the S forms were highly unstable on this medium. By exposing suspensions of the selected

TABLE II

EFFECT OF TREATING TWO-WEEK-OLD CULTURES OF BOVINE 599 PREVIOUSLY TREATED WITH X-RAYS TO A SECOND IRRADIATION FOR 12 MIN.

Culture	Subculture					Type of colony
	1	2	3	4	5	
Control	37	33	50	40	50	% R
	50	19	10	20	5	% S
	13	50	40	40	45	% RS
X-rayed	5	3	7	10	0	% R
	20	45	93	90	100	% S
	75	52				% RS

colony to X-rays, before plating, as in the previous experiments, the isolation of S types was facilitated. In a representative instance an apparently S colony was suspended and a sample plated; from this plate a second apparently S colony was selected, and so on through five culture generations with the indifferent results indicated in the control, Table II. A similar colony apparently S was suspended but irradiated before plating, and from this plate a second apparently S was suspended in fluid and X-rayed and this repeated through five culture generations. The results shown in Table II indicate a much higher yield of S than in the non-radiated control. Comparable results were obtained in several instances.

III. Differences in Stability of R Colonies

In the previous paper concerning this organism, it was shown that when R colonies, apparently similar, were selected and exposed to the same cultural conditions they did not exhibit the same degree of variability; some continued to produce only R colonies, others produced S colonies and intermediate colonies at varying rates. The same was shown in Section II to be true of X-rayed suspensions. Gates (5) found that apparently similar bacteria varied in respect to their sensitivity to ultra-violet light, and similar observations have been made in respect to many agencies. This might be explained on the

TABLE III

EFFECT OF TIME OF EXPOSURE TO X-RAYS UPON CULTURES OF BOVINE 599

Culture	Time, min.	Strength of current, milliamperes	Dosage, m. amp. min.	Organisms per cc. of emulsion	Per cent killed
Control	—	—	—	1,696,000	—
X-rayed	5	3	15	840,000	50
X-rayed	10	3	30	525,000	69
X-rayed	15	3	45	382,000	78
X-rayed	20	3	60	296,000	83

assumption of different degrees of fixity in the individual organisms of those characteristics which contribute to the visible form of the colony. The difference might result from the fact that the colony capable of producing variability contains two or more types of individual organisms, and as in plating one or more of the types might not be included, their characteristic colony type would therefore not appear on the plates. The latter suggestion would appear to be inadequate to explain the fact noted in the previous paper that over a period of two years this organism exhibited conspicuous variability. Two years later the R strains, which had been carried during this period by transferring every one to two months on slants of gentian violet-egg media, appeared to have become perfectly stable and exhibited no dissociation in fluid media in which they had previously dissociated. At this period the X-ray treatment which had earlier promoted variability, failed to produce any change in the stability of the R forms.

IV. The Influence of Dosage of X-rays

Several experiments have indicated that the dosage used is one of considerable toxicity. Suspensions of R forms were exposed to various intensities of X-rays and plate counts made of the surviving bacteria. Table III indicates the results of varying the time of exposure and Table IV of varying the time and the amperage. In both cases the voltage remained at approximately

TABLE IV

THE EFFECT OF THE INTENSITY OF IRRADIATION UPON CULTURES OF BOVINE 599

Culture	Strength of current, m. amp.	Fall in potential, volts	Time, min.	Dosage, m. amp. min.	Number of organisms per cc.	Per cent killed
Control	—	—	—	—	428,000	—
X-rayed	2	88,000	10	20	129,000	70
X-rayed	4	82,000	5	20	137,000	68
X-rayed	3	84,000	10	30	108,000	75
X-rayed	4	82,000	10	40	43,600	90
X-rayed	5	79,000	10	50	14,000	97

88000. It is apparent from the tables that the lethal effect is considerable and that it is proportional to the dosage. Applying these dosages to R strains, which exhibited no dissociation in successive cultures in alkaline broth,

resulted in no change in the form of the colonies developing from the surviving organisms.

V. The Effect of Protective Colloids

McKinley and Fisher (18) found that the presence of normal rabbit serum protected bacteriophage and viruses from the lethal effects of ultra-violet light. Meyer (15) found a similar lack of bactericidal action in the presence of haemoglobin. Harris and Hoyt (8) investigated the absorption capacity of a number of substances and found that aromatic radicals possessed this property to a marked degree. Eidinow (4), in studying the bactericidal action of light, found that bacilli mixed with blood and exposed in a very thin film or in a mixture of defibrinated rabbit blood were not killed in one hour.

In order to determine the protective effect, if any, of serum and gelatine, these substances were added to a suspension of the *Mycob. tuberculosis bovis* No. 599 organisms, known to consist of both R and S types. They were then exposed to the dosage of X-rays used in the previous experiments. The results are shown in Table V. In the radiated preparation without protein, as in the former cases quoted, the proportion of S types was increased over the unexposed control. Where either 1% gelatine or 1-10 blood serum was added,

TABLE V

THE EFFECT OF PROTECTIVE COLLOIDS UPON THE SENSITIVITY OF BOVINE 599 TO X-RAY TREATMENT

Culture	Protein	R colonies %	S colonies %	Intermediate colonies %
Control	0	34	66	0
X-rayed	0	10	86	4
Control	Gelatine	21	75	4
X-rayed	Gelatine	26	58	16
Control	Blood serum, 1-10	41	59	0
X-rayed	Blood serum, 1-10	24	66	10
X-rayed	Blood serum, 1-10	35	50	15

however, there was comparatively little difference between the unexposed control and the irradiated preparations. The protein apparently protected the bacteria against the action of the X-rays.

The Effect of X-rays upon Other Species of Bacteria

Meyer (15) reported that tubercle bacilli were not more sensitive to ultra-violet light than other species of bacteria. But as others have pointed out it is probably inaccurate to draw analogies between the effects of different rays, though Meyer (15) demonstrated that cultures resistant to ultra-violet were also resistant to X-rays. Browning and Russ (1) and Gates (5) have shown a definite relationship between the bactericidal effect of light and the absorption spectrum of the bacterial emulsion; Meyer (15) suggests that this may be due to differences in amino complexes in the different organisms.

Several strains of recently isolated *Es. coli* and *Staph. aureus* were exposed to the same dosage of X-rays as used in the previous experiments. No reaction to this X-ray dosage was observed.

A culture of paratyphoid bacilli which had been under observation for a considerable time and which was known to include both R and S colony types was suspended in saline and exposed to the dosage of X-rays used in the previous experiments with the tubercle bacilli and to double this dosage. Plates made from the suspensions before and after irradiation exhibited the same proportion of R and S forms. It seems apparent therefore that an X-ray dosage which does affect certain acid-fast bacteria is without influence upon these representative non-acid-fast bacteria.

Discussion

Several theories as to the action of light upon living matter have been brought forward. Clark (2) developed a photochemical theory postulating that electrons are given off by cell proteins under the influence of ultra-violet light and that these attach themselves to other atoms and molecules with resulting changes in the physical and chemical properties of all the substances concerned. Mayer (14) outlined a very similar explanation; the photochemical rays are absorbed by the reacting substance with a resultant increase in electron activity and liberation of electrons. Since most living particles are negatively charged, this would bring about a gain in positive electricity. Gutfeld and Pincussen (7) also attribute the photochemical effects of light to a loss in negative dispersion and definite physiological disturbance. Hill (10) and Lacassagne and Holweck (12) make somewhat similar suggestions. The inactivation of trypsin by irradiation according to Clark and Northrop (3) seems also to depend upon electrical neutralization. The early work of Young (25) on the reduction of potential in bacterial suspensions after irradiation agrees with these theories.

These observations are very suggestive in view of the finding of several observers that variant types of bacteria exhibit differences in electrical charge. The work just reported by Kahn and Schwarzkopf (11) in which it is shown that S types of avian and bovine tubercle bacilli possess a higher negative charge than the R types is especially interesting in this connection. Work along this line is now in progress and will form the subject of a further paper in this series in the near future.

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